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Intruders below the Radar: Molecular Pathogenesis of *Bartonella* spp.

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INTRODUCTION

The genus *Bartonella* constitutes a group of facultative intracellular pathogens that share a unique stealth infection strategy aiming at persistence in an intraerythrocytic niche, thus enabling continuous transmission by bloodsucking arthropods and establishing a sanctuary protected from the assault of the host's immune system.

Each of the bartonellae infects only one or a few closely related mammal species as its reservoir host, a relation defined by the capability of *Bartonella* to establish an intraerythrocytic and typically asymptomatic bacteremia, although infections in incidental hosts may evoke discernible disease. Several species of the genus *Bartonella* are human pathogens, including both zoonotic agents and species that infect humans as their reservoir host. While most human infections, for example, with *Bartonella henselae* or *Bartonella quintana*, elicit only limited morbidity, *Bartonella bacilliformis* is unique as a deadly pathogen causing Carrion's disease, which can kill more than 80% of the patients during its acute phase but is restricted to the Andes region (reviewed in reference 272). Consistent with this, phylogenetic analyses identified *B. bacilliformis* as the only known representative of an ancient lineage that is widely separate from the other, so-called modern species of the genus (374).

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Despite very limited public awareness of *Bartonella* outside the areas where *B. bacilliformis* is endemic, research over the last 20 years has uncovered the virtual ubiquity of *Bartonella* bacteremia among mammals, thus justifiably tempting other researchers to question the typical notion of blood as a sterile organ (51). Infection rates of higher than 50% are common among wild-living mammals, and, e.g., 15 to 30% of pet cats are infected, thereby constituting a large reservoir for zoonotic *Bartonella* infections that can cause considerable morbidity, especially in immunocompromised patients (see below). The bartonellae are currently classified as (re)emerging pathogens, since various species are increasingly found to elicit human disease, most likely due to changes in human society and medical practice but also thanks to improved diagnostics (46, 220, 301).

We are convinced that investigating the molecular and cellular bases of *Bartonella* infections *in vitro* and *in vivo* is crucial for understanding the pleiotropic pathology in human patients, because bacterial virulence strategies evolved not to cause disease in the host but to promote continuous spread and proliferation. It is therefore necessary to set the results from molecular and cellular microbiology into context with clinical as well as veterinary studies in order to understand the infection process as a whole. However, the assembly of a comprehensive picture is hampered by the obstacle that, as already stated by others, the bartonellae remain understudied (294) compared to more famous pathogens, and as a consequence, available knowledge often appears to be fragmented. This review therefore aims at compiling published work on the molecular pathogenesis of *Bartonella* and tries to highlight the various links to medical research in the field.

EPIDEMIOLOGY AND CLINICAL ASPECTS

Epidemiology

Striking evidence for the success of the bartonellae as stealth pathogens is that the majority of species have been discovered only very recently, although *Bartonella* infections are virtually ubiquitous among mammals (90). Remarkably, the infection rate may reach up to around 50% in feral cats or rodents and can be as high as 90% in wild ruminants (reviewed in reference 51). Apart from these groups of thoroughly investigated animals, *Bartonella* infections have been reported for diverse hosts such as bats (240), aquatic mammals (belugas [270]), and even nonmammal vertebrates (sea turtles [423]). Despite certain geographic differences that coincide with vector ecology, it is obvious that *Bartonella* infections are among the most prevalent bacterial infections worldwide.

Although the ubiquity of bartonellae in various mammals is an important reservoir of zoonotic infections, the majority of human disease is caused by only three species. *B. henselae*, whose reservoir host is the domestic cat, is well known to be the most common species infecting humans and characteristically causes cat scratch disease, a condition associated with only limited morbidity (see below). It was estimated at the end of the 20th century that 22,000 cases of cat scratch disease may appear every year in the United States, and roughly 10% of these were considered to require hospitalization (202). *B. quintana* is the only modern species of *Bartonella* that infects humans as its reservoir host, and it is known to cause trench fever, a louse-borne disease that was widespread in armies of the modern era such as Napoleon's Grand Army (350) and affected approximately 800,000 allied soldiers in France during World War I (66). The incidence of trench fever has dropped considerably since then, but infections with *B. quintana* are (re)emerging as urban trench fever, a condition that is associated with homelessness, alcoholism, indigence, and poor conditions of life, particularly health and hygiene, among individuals living on the fringes of society in developed countries (59, 189, 203, 399).

In contrast to the other species of the genus, *B. bacilliformis* is a deadly pathogen but endemic only in the high valleys of the Andes and apparently restricted to infecting humans (recently reviewed in reference 272). It can cause devastating disease and has been known as a pathogen among natives since the pre-Incan times (5) but apparently became a general health concern only with alterations in epidemiology due to climate change and increased migration into and out of the areas of endemicity in the last 150 years (81, 87, 131, 239). Disease caused by *B. bacilliformis* first received major attention around 1870 during the construction of the Oroya railroad through a region of endemicity, as approximately 70% of the (mostly nonlocal) workers died, to a large extent from the hemolytic anemia that is known today as Oroya fever (325). The seroprevalence of *B. bacilliformis* can be higher than 60% in natives of the areas of endemicity (230), and a considerable proportion of around 10% of the population was found to bear asymptomatic bacteremia, thus probably serving as a reservoir for infection (see various studies listed in reference 325).

Clinical Aspects

The most common malady associated with *Bartonella* in immunocompetent patients is cat scratch disease due to infection with *B. henselae*, a condition characterized by regional lymphadenopathy as the leading symptom, which may be accompanied by other unremarkable manifestations such as fever or fatigue (reviewed in reference 249). Cat scratch disease is usually self-limiting, though possibly long lasting, and can occasionally be caused by other *Bartonella* species such as *B. clarridgeiae* (237). Infections of immunocompetent individuals with *B. quintana* typically lead to a disease known as trench fever or 5-day fever, denoting the characteristic cyclic course of the symptoms with peaks of fever, bone pain, and headache that are accompanied by persistent bacteremia (66, 318). Both *B. quintana* and *B. henselae* are further able to elicit bacillary angiomatosis, i.e., the outgrowth of multiple vasoproliferative tumors, as a common complication of infection, primarily but not exclusively in immunocompromised individuals such as AIDS patients. While these lesions are most often found on the skin, bacillary angiomatosis or related conditions may also affect the liver ("bacillary peliosis"), spleen, bone marrow, eyes, or other parts of the body (304, 339). The dissemination of *Bartonella* infections is a complication observed mostly in highly immunocompromised patients, but it can also occur in apparently immunocompetent individuals. Such systemic infection not only with *B. henselae* or *B. quintana* but also with various other zoonotic species can lead to a plethora of symptoms, including extraerythrocytic bacteremia, neuroretinitis, or endocarditis (55, 94, 143). In addition, various moderate neurological symptoms such as insomnia or memory loss have been reported (53, 56, 73).

The classical course of infections with *B. bacilliformis* is known as Carrion's disease and consists of Oroya fever as the acute phase with hemolytic anemia and a subsequent chronic phase hallmarked by multiple vasoproliferative lesions on the skin (verruga peruana for "Peruvian wart") (Carrion's disease was recently reviewed in reference 272). The devastating hemolytic

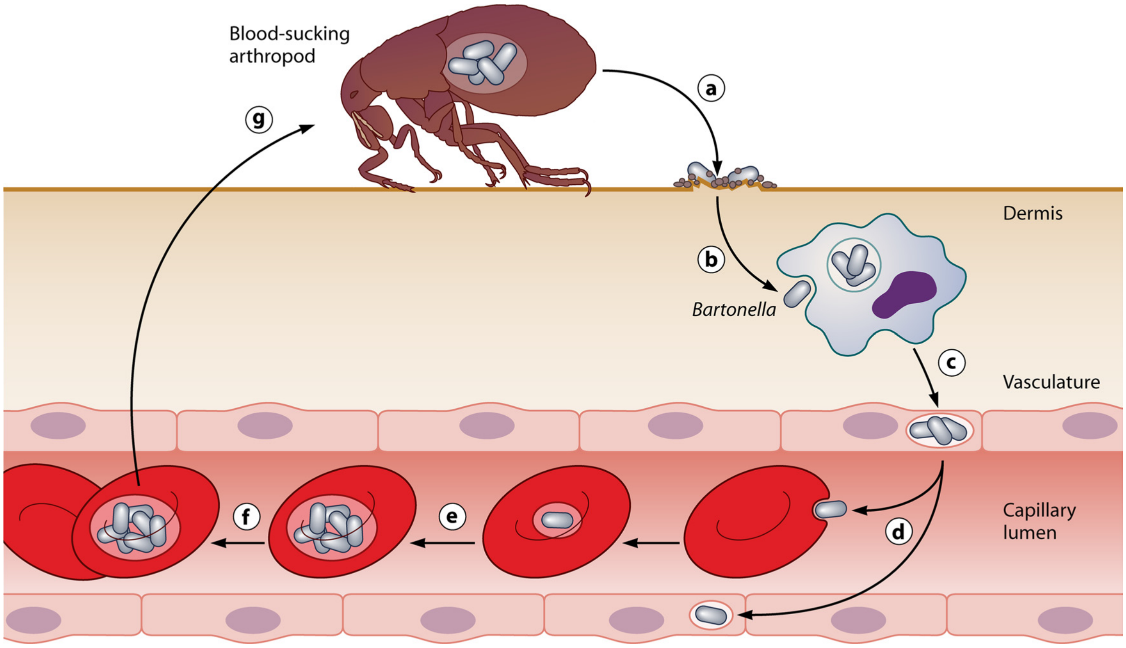


FIG 1 Common infection strategy of the bartonellae. The drawing illustrates the general concept of reservoir host infections with *Bartonella*. Following transmission by an arthropod vector (a), the bartonellae colonize the primary niche, which probably involves entry into migratory cells (b) and transport to the vascular endothelium (c), where the bacteria persist intracellularly. From the primary niche, the bacteria are seeded into the bloodstream (d), where they invade erythrocytes and reinfect the primary niche. After limited replication inside the red blood cell (e), they persist in the intraerythrocytic niche (f) competent for transmission by a bloodsucking arthropod (g).

anemia during Oroya fever is a critical difference between the pathologies of *B. bacilliformis* and the other bartonellae, since severe morbidity and hemolytic anemia are usually not observed during infections with the modern species. A recent compilation of various studies concluded that the mortality during Oroya fever ranges from close to zero in the case of hospitalized patients receiving antibiotic treatment to up to 88% in untreated cases (325). Approximately 70% of patients suffering from Oroya fever develop complications of the disease, of which approximately half are of an infectious and half of a noninfectious nature. Noninfectious complications such as fever, anorexia or an altered mental status have been suggested to be primarily a consequence of systemic infection (271). The impairment of immunity during Oroya fever favors secondary infections that contribute prominently to morbidity and mortality by provoking septicemia, most often with *Salmonella* (271, 325, 359). During Carrion's disease, the abrogation of hemolytic anemia is characteristically followed by massive pathological angiogenesis as hallmark of the chronic tissue phase called verruga peruana. It is crucial to note that the nodules observed over the course of this phase are "clinically and histologically indistinguishable" from the tumors arising during bacillary angiomatosis with the modern *Bartonella* species (67, 254). Like these, the pathological angiogenesis during the verruga peruana phase usually occurs in the skin, but atypical cases involving other organs such as the spleen (266) have been reported. In striking contrast to the acute phase of Carrion's disease, verruga peruana is associated with only negligible mortality (325). Notably, the name of this biphasic infection and the discovery of the link between its two phases derive from Daniel Alcides Carrion, who died from Oroya fever after he inoculated himself with blood from a verruga peruana nodule (396). However, it is known today that this classical course of Carrion's disease is not the only possible outcome of a *B. bacilliformis* infection, since natives in the areas of endemicity exhibit a significant baseline of asymptomatic bacteremia (see above) and suffer from Oroya fever relatively rarely (359) but frequently develop verruga peruana without prior hemolytic anemia (150).

LIFE CYCLE AND INFECTION STRATEGY

The infection cycle of *Bartonella* is initiated with the inoculation of a mammal reservoir host, characteristically following transmission via bloodsucking arthropods. Upon inoculation, the bartonellae are not capable of directly colonizing erythrocytes. Instead, a preceding period of residence in a primary niche is apparently necessary to make the bacteria and/or the host competent for this step. From the primary niche, the bartonellae are seeded into the bloodstream and infect erythrocytes in a sequence of steps ranging from adhesion to eventually invasion and intracellular persistence that enables continuous vector transmission (Fig. 1).

During the whole course of infection, the lack of an effective host immune response and a globally moderated inflammatory profile are highly beneficial for *Bartonella* and are promoted by the pathogen via both passive immune evasion and active immunomodulation. The infection cycle of *Bartonella* has been extensively studied and reviewed elsewhere (see references 89 and 403). Importantly, although it is obvious that certain differences from species to species do exist and probably arose due to specific paths of pathogen-host adaptation, it is generally believed that the overall concept of this infection cycle is conserved among members of the genus *Bartonella*.

Host Specificity

It is apparent from the epidemiology of *Bartonella* infections that host specificity must be a determining factor for the selective prevalence of these bacteria in various mammals (51, 121). Furthermore, the multistep infection strategy of *Bartonella* suggests that host specificity could be a complex phenomenon, because every step may involve critical interactions of the bacterium with its hosts that could require fine-tuned manipulations on the molecular level.

Since inoculation of a naive host individual with *Bartonella* classically requires transmission via a bloodsucking arthropod, it is obvious that vector ecology has a major impact on host specificity. However, direct transmission between mammal hosts has occasionally been reported, e.g., via cat bites (57), and the release of *Bartonella* into the saliva of infected cats has been demonstrated (311).

It is well known that the sand fly (*Lutzomyia verrucarum*) is the most important vector for *B. bacilliformis*, and the geographic range of *L. verrucarum* prevalence is roughly congruent with the areas where Carrion's disease is endemic (325). However, certain differences in the distributions as well as recent epidemics in areas outside the geographic range of *L. verrucarum* indicate that other *Lutzomyia* species may serve as additional vectors (4, 68, 325). The role of human body lice (*Pediculus humanus corporis*) and their feces in the transmission of trench fever was well known already more than half a century before the discovery of *B. quintana* (66), but other vectors such as cat fleas (*Ctenocephalides felis*) have been occasionally reported as well (368). Cat fleas are the natural vector of *B. henselae*, and their potency to transmit the pathogen between cats has been demonstrated experimentally (93). Transmission to humans is thought to occur mostly indirectly via contaminated flea feces that are inoculated via a cat scratch ("cat scratch disease") (89). It was shown for both human body lice and cat fleas that *B. quintana* and *B. henselae*, respectively, can replicate in their digestive systems and are shed in the arthropod feces (139, 145, 398). More general, it is obvious that other hematophagous arthropods are capable of transmitting *Bartonella* as long as the bacteria can survive within these vectors and make productive contact with new host individuals. For example, the long-debated capability of ticks to serve as vectors for *Bartonella* has recently been demonstrated (354), and biting flies are known to play an important role in the transmission of *Bartonella* among ruminants where they are predominant bloodsucking parasites (122, 279). Coincident with the prevalence of the common arthropod vectors, the *Bartonella* infection rate among mammals is usually higher in warm, humid areas than in cold, arid ones (29, 206) and higher in wildlife than in domestic animals (e.g., 15 to 30% in pet cats [91, 166, 167] compared to up to 50% in feral cats [173]). The relationship between *Bartonella* and the different vectors has been comprehensively reviewed elsewhere (89).

It is striking that the effects of vector ecology via selective transmission are not at all sufficient to explain current knowledge on the host specificity of *Bartonella*, especially since host specificity has been readily reproduced in experimental infections under laboratory conditions in the absence of any vector. In particular, persistent bacteremia as the hallmark of reservoir host infection was obtained only when using reservoir host models (134, 165, 233, 236, 242, 330, 394). Attempts to infect nonreservoir hosts with bartonellae resulted neither in verifiably intraerythrocytic bacteremia nor in persistent infection (92, 134, 195, 213). However, it is known that the host range of *Bartonella* does not strictly follow the taxonomic borders on the species level but may include a few closely related species, so that *B. quintana* has been reported to infect not only humans but also rhesus macaques, both naturally and upon experimental inoculation (190, 450). Since the *Bartonella* infection strategy involves complex interactions with both nucleated host cells and erythrocytes, it is apparent that host specificity can act on both of these levels. Strong evidence supporting an intricate host species/host cell type/*Bartonella* species specificity concerning phenotypes associated with the primary niche (see below) points toward a certain restriction already at this level, probably eliminating a considerable number of incidental infections due to improper immunomodulation. In addition, the establishment of intraerythrocytic bacteremia as the key step to enable continuous transmission is known to be critical for host specificity of the modern bartonellae. It has been demonstrated that the ability of these species to adhere to erythrocytes is highly restricted to those of reservoir hosts, while this step is apparently not limiting for *B. bacilliformis* to a similar extent (see "Infection of Erythrocytes" below). However, no considerable pathology of any experimental infection of nonprimates with *B. bacilliformis* has been reported (see references 195 and 313), strongly suggesting that further mechanisms in addition to vector ecology restrict the host range of this deadly pathogen.

The Primary Niche

Cellular nature of the primary niche. Little *in vivo* evidence is available that would allow us to draw a precise picture of the infection stage between the inoculation of *Bartonella* into the skin (e.g., from the feces of arthropod vectors) or the bloodstream (e.g., from ticks or sand flies) and the bacteremic stage of the infection. Intravenous inoculation of *Bartonella* does not lead to immediate infection of red blood cells (see below), strongly suggesting that the bacteria and/or the host needs to be primed in some way to enable erythrocyte colonization and implying that *Bartonella* persists in a primary niche prior to blood-stage infection (394). While the nature of this niche in the case of infections with the modern species constitutes one of the key controversies in the field, almost no research has been performed on the primary niche in infections with *B. bacilliformis*. However, the existence of such a niche for this species is widely accepted (271, 417) and may explain part of the remarkably long incubation time of Oroya fever (60 days on average [272]).

No conclusive evidence that would unambiguously reveal the cellular identity of the primary niche has been published. However, the abundance of accordant clinical conditions, the amount of *in vitro* data supporting extensive interactions with the pathogen, and their obvious proximity to the circulation have established endothelial cells as an important target of *Bartonella in vivo* and a likely candidate to be part of the primary niche (reviewed in reference 113). However, it is likely that additional cell types are involved in this niche and bring in properties or abilities that cannot be supplied by endothelial cells. Most obvious is that the apparent need for bacterial transport within the host (e.g., from typical intradermal inoculation) implies that migratory cells may play an underestimated role in the establishment of *Bartonella* infections. It seems reasonable to assume that the bartonellae could enter migratory cells after inoculation and travel passively to a remote location in the host's body where they would persist and multiply, most probably in the microvasculature. The frequent affliction of lymph nodes during *Bartonella* infections may indicate that such transport might occur via the lymphatic system, and it has been proposed that lymphocytes or mononuclear phagocytes could be the vehicles of *Bartonella* transport as part of the primary niche (236). The long life span of these cells and their circulatory migration between tissues and vasculature not only may be part of the classical course of *Bartonella* infection but also could promote the dissemination of the pathogen in the host's body as a common complication of cat scratch disease (see above). A possible role of migratory cells as part of the *Bartonella* infection strategy is striking, considering that *B. henselae* can resist macrophage killing for at least 3 days (236) and establish an intracellular niche in these cells that is distinct from the endocytic pathway (246). Consistent with this, a murine model of *B. henselae* infection revealed that bacteria are detectable in liver and lymph nodes within 6 h after intraperitoneal inoculation (219), which is difficult to explain without transport of the pathogen by a host mechanism.

Other studies proposed hematopoietic progenitor cells or erythroblast cells, nucleated progenitors of erythrocytes, as part of the primary niche (162, 274, 366). Since mature red blood cells are nonendocytic (see below), it is an interesting hypothesis that *Bartonella* could infect erythrocyte progenitors and then enter the bloodstream coincident with their maturation. However, it has been demonstrated for *B. tribocorum* that *Bartonella* can invade mature red blood cells *in vivo* (394). It is therefore not necessary to assume that erythrocyte progenitors are part of the primary niche, although it seems clear that *Bartonella* can infect these cells *in vivo* and that they, when mobilized, may contribute to (re)infection of the bloodstream as well as dissemination of the pathogen, as suggested by others (376).

The frequent relapse of *Bartonella* infections in reservoir and incidental hosts after antibiotic treatment and/or apparent clearance of bacteremia by the immune system (examples in references 66, 155, 236, and 339) indicates that the bacteria in the primary niche and potentially in disseminated niches would be protected from such assault, for example by persisting intracellularly from where they may reinfect the bloodstream. A periodic seeding of bacteria from the primary niche into the circulation at intervals of several days has been demonstrated in the *B. tribocorum* rat infection model (403) and may very well explain the cyclic relapse of symptoms ("5-day fever") that is the hallmark of *B. quintana* infection in humans (66, 114). However, it is not clear if such regularity is a general feature of *Bartonella* infections.

Intracellular bartonellae in nucleated cells *in vivo* have been frequently reported, e.g., *B. bacilliformis* in littoral cells lining lymphatic sinuses (359) or *B. quintana* in the cardiac endothelia of patients suffering from infective endocarditis (60, 351), although the relationship of these compartments to the primary niche is unclear. A number of *in vitro* studies showed that *B. henselae* can invade diverse cell types such as endothelial cells (120), endothelial progenitor cells (376), epithelial cells (24), hematopoietic progenitor cells (274), monocytes/macrophages (223, 309) (including microglial cells [307]), and even tick cells (40). The ability to manipulate such a variety of cell types is very likely to contribute to the highly diverse illness with a plethora of different symptoms that can arise in human patients suffering from systemic cat scratch disease. A wide range of different cell types have also been found to be invaded by *B. bacilliformis*, including HeLa cells as well as human dermal fibroblasts, human laryngeal epithelial cells, and human umbilical vein endothelial cells (HUVECs) (182).

Interaction with nucleated host cells. The interaction of *Bartonella* with nucleated host cells (particularly endothelial cells) has been thoroughly studied *in vitro*, revealing a set of leading phenotypes: cellular invasion, activation of NF-κB and HIF-1 signaling, inhibition of apoptosis, and mitogenic stimulation (360, 385). Though likely to play a key role in the colonization of the primary niche, it is obvious that the underlying mechanisms will also be involved in the colonization of secondarily infected sites, which may be identical to the primary niche or feature additional cell types, especially during disseminated infection at various sites of the host's body. Several virulence factors of *Bartonella*, such as the trimeric autotransporters or the VirB/D4 type IV secretion system (T4SS), have been shown to participate in the interaction with nucleated host cells, and it appears as if they would not contribute to strictly separated aspects of infection but rather would manipulate a set of cellular responses together (Table 1).

(i) Cellular invasion. The entry of *B. henselae* into human endothelial cells can occur via two mutually exclusive pathways, either as single bacteria using an apparently zipper-like mechanism (224) or in form of large aggregates in a remarkable structure that is known as the invasome (120). The uptake of single bacteria or small conglomerates in the zipper-like mechanism yields *Bartonella*-containing vacuoles (BCVs) that fail to acidify and to fuse with lysosomes but instead accumulate in the perinuclear space (246). Residence in perinuclear vacuoles was also reported for *B. bacilliformis* (429) and *B. quintana* (60, 395), indicating that this mode of intracellular persistence may be a common property of the bartonellae and possibly reflect a cellular niche colonized during infection *in vivo*. The entry of *B. bacilliformis* was found to be fully or partially dependent on the activation of the small GTPases Rho, Rac, and Cdc42, on an altered pattern of tyrosine phosphorylation, and on $\alpha5\beta1$-integrins. Furthermore, the invasion process was dependent on a bacterial surface protein and involved actin rearrangements that manifested, during the course of intracellular persistence, as a massive formation of stress fibers anchored to focal adhesions (182, 428, 439). Interestingly, the intracellular vacuoles colonized by this species were shown to be associated with the Golgi complex, whose functions the pathogen could possibly manipulate in its favor, although intracellular replication has not been demonstrated (429, 430).

Because invasome formation has been the main focus of studies investigating the entry of *B. henselae* into host cells (see below), much less is known about the zipper-like uptake process and, in particular, if it is directly or indirectly related to the uptake of *B. bacilliformis*. One study suggested that *B. henselae* would replicate inside human endothelial cells, and the inhibition of host cell protein synthesis with cycloheximide seemed to impair intracellular replication, indicating that the pathogen would obtain nourishment from resources supplied by the host cell metabolism (224). Consistent with this, the stimulation of endothelial cell proliferation via vascular endothelial growth factor (VEGF) significantly raised the growth rate of *B. henselae* during experimental infection (225). In apparent congruence to the findings with *B. henselae* and *B. bacilliformis*, *B. quintana* was shown to interact with membrane ruffles and to invade endothelial cells within 1 min, where it then divides in intracellular vesicles (60).

(ii) Pathological angiogenesis. Bacillary angiomatosis and verruga peruana lesions are key elements of the pathology related to

TABLE 1 Virulence factors of *Bartonella* spp.

Virulence factor(s)	Direct function(s)	Contribution(s) to pathogenesis
Trimeric autotransporter adhesins (BadA, Vomps)	Bacterial autoaggregation, attachment to extracellular matrix, host cell binding via β1-integrins, activation of HIF-1 and NF-κB, inhibition of macrophage phagocytosis	Stable interaction with host cell as basis for further manipulation, secretion of proangiogenic cytokines
VirB/D4 type IV secretion system, *Bartonella* effector proteins	Inhibition of apoptosis, proinflammatory activation, modulation of angiogenesis, invasome formation	Setup and control of intracellular niche
Unknown factor (probably surface protein)	Activation of cellular signaling via β1-integrins	Triggering host cell invasion in course of invasome formation
Outer membrane proteins	Activation of NF-κB signaling, host cell adhesion	Secretion of MCP-1 and upregulation of E-selectin and ICAM-1 (as part of proangiogenic signaling?)
Secreted factor (GroEL)	Unknown, activation of Ca^{2+} signaling?	Inhibition of apoptosis and mitogenic stimulation of host cells
Trw type IV secretion system	Adhesion to erythrocyte surface	Enabling erythrocyte invasion (lineage 4)
Deformin	Formation of invaginations in erythrocyte membranes	Enabling erythrocyte invasion
Flagellation	Mechanical force?	Invasion of erythrocytes (lineages 1-3)
Invasion-associated locus (IalAB)	Unknown	Invasion of erythrocytes
Lipopolysaccharides	Invisibility to TLR4, antagonizing TLR4 signaling	Immune evasion and immunomodulation
Autotransporters	Unknown, cohemolysin activity (Cfa)?	Unknown
Filamentous hemagglutinins	Unknown	Unknown
Hemin binding proteins	Hemin binding, fibronectin binding (HbpA)	Host cell adhesion?, formation of a heme surface layer (antioxidant barrier? nutritive reservoir?)
OMP43	Fibronectin binding, host cell binding	Host cell adhesion?

Bartonella in human patients and appear as macroscopic symptoms corresponding to the remarkable angiogenic activities of these pathogens that have been detected *in vitro* (113, 150). Histologically, the pathological angiogenesis during *Bartonella* infection results in tumor-like lesions packed with lobes of immature capillaries that are lined with a swollen endothelium. Bacterial aggregates as well as dispersed bacteria can be found in close proximity to the proliferating endothelium, and the lesions are characteristically infiltrated by phagocytes such as macrophages and neutrophils (39, 97, 273). Maintenance of these peculiar tumors is fully dependent on the presence of live bacteria, since eradication of the pathogen by antibiotic treatment is known to efficiently resolve the lesions (365). The striking similarity of clinical and histological features and the strong association of both bacillary angiomatosis and verruga peruana with preceding immunosuppression generated the hypothesis that these conditions may be caused by similar means of molecular pathogenesis (86).

The angiogenic properties of *Bartonella* can be subdivided into a direct mitogenic and antiapoptotic stimulation of endothelial cells and the triggering of autocrine and paracrine cytokine secretion that synergizes with the direct effects. Current knowledge indicates that the angiogenic properties of *B. henselae* proceed through at least two apparently distinct pathways, one via a secreted factor that stimulates Ca^{2+} signaling (283) and another via a certain proinflammatory activation involving NF-κB that is possibly contact dependent (223). Similarly, a secreted mitogen or antiapoptotic factor as well as an activation of NF-κB has been reported for endothelial cells infected with *B. bacilliformis* (297, 430). However, most studies did not separate secreted and contact-dependent activities, and it is very likely that the results under normal infection conditions *in vitro* reflect the cumulative effect of both.

Histological analysis of verruga peruana nodules revealed that *B. bacilliformis* infection stimulates the production of VEGF, its receptors VEGFR-1 and VEGFR-2, and angiopoietin-2 in host cells (80). Immature vasculature is known to require a constant supply of VEGF as a survival factor (30), and VEGF and angiopoietin-2 together have a strong, synergistic effect on angiogenesis that is linked to both mitogenic and antiapoptotic activities (264, 445). Remarkably, the endothelium in the verruga peruana lesions was found to produce all of these factors except VEGF, which seemed to be supplied by the surrounding epidermis, suggesting that vascular tumor formation during verruga peruana would be driven by the cooperation of several cell types (80). The secretion of VEGF has also been demonstrated in vasoproliferative tumors that arose during human infection with *B. henselae* (225), and an angiogenic mechanism involving other cell types in addition to endothelial cells is strongly supported by published work on the modern species as well.

The infection of human endothelial cells with the two most prevalent lab strains of *B. henselae* induces a prominent transcriptional reprogramming that involves the activation of inflammatory pathways and leads to elevated expression levels of various factors supporting angiogenesis, such as ICAM-1, angiopoietin-2, interleukin-8 (IL-8), or Bcl-2-related protein A1 in response to the Houston-1 typing strain (ATCC 49882^{T}) (124) and a closely related set of genes in response to the Marseille strain (see below). Remarkably, although both strains also induced a significant transcriptional upregulation of VEGF, neither the Houston-1 nor the Marseille strain was able to trigger any considerable secretion of VEGF in infected endothelial cells (124, 225). These results are in contrast to the fact that VEGF was detected in bacillary angiomatosis tumors *in vivo* (225) and thus confirm that other cell types in the vasculoproliferative lesions would produce VEGF to drive angiogenesis. However, massive secretion of IL-8, a potent proangiogenic cytokine, was readily detected in endothelial cells upon infection with *B. henselae* (284). Further experiments in the same study revealed a critical role of autocrine IL-8 signaling in the stimulation of endothelial cell proliferation and angiogenic phenotypes in response to *B. henselae* infection. The mitogenic stimulus was accompanied by a potent antiapoptotic effect via a strong increase in the ratio of Bcl-2 to Bax, and both cell proliferation and inhibition of apoptosis were dependent on IL-8, thus confirming published work that IL-8 heavily promotes angiogenesis by stimulating endothelial cell proliferation and inhibiting apoptosis via pathways including the aforementioned ones (257). It is also known that an upregulation of Bcl-2 can stimulate IL-8 secretion, suggesting that positive feedback may already play a role in this autocrine signaling (315), especially since *B. henselae* infection also triggered an increase in surface expression of an IL-8 receptor (284). As expected from the clinical similarity, both Bcl-2 upregulation and a parallel mitogenic effect have also been observed in endothelial cells infected with *B. quintana* (258). However, it is apparent that the investigation of endothelial cells infected with bartonellae *in vitro* does not fully represent the processes driving pathological angiogenesis *in vivo*, because VEGF secretion is missing.

In contrast to the results with endothelial cells, stimulation of VEGF secretion upon infection with *B. henselae* was readily detected in several other cell types, such as epithelioid or monocytic cells and macrophages (i.e., Mono Mac 6 cells) (223) and HeLa cells and Ea.hy926 cells (222); the latter were derived from a fusion of HUVECs with epithelial cancer cells and thus exhibit characteristics of both cell types (129). The secretion of VEGF from HeLa cells and human THP-1 macrophages was also evident upon infection with *B. quintana* (395). Although inhibition of apoptosis was apparent in monocytes, it was contact dependent and driven via NF-κB (223), suggesting that the secreted factor could not act on these cells. *B. henselae* further failed to inhibit apoptosis in human fibroblasts or epithelial cell-like dog kidney cells (MDCK), and although various strains of *B. henselae* as well as *B. quintana* inhibited apoptosis and stimulated proliferation of human endothelial cells, no such effect was detected in the parallel examination of *B. clarridgeiae*, *B. elizabethae*, or *B. vinsonii* (226). Unfortunately, the authors of that study did not test endothelial cells of species other than humans, but they conclusively suggested a certain host species/cell type/bacterium specificity as underlying the differential effects they observed. Importantly, these results are in line with other work reporting that *B. bacilliformis* extracts contained a mitogenic activity specific for endothelial cells (150). A recent study confirmed the relevance of host species and cell type by showing that *B. henselae* was unable to stimulate the proliferation of various feline cell lines, in contrast to the case for several human lines (37). Furthermore, the authors reported that human microvascular and macrovascular endothelial cells responded differently to infection with *B. henselae* (37). It is important to realize that vascular tumor formation *in vivo* is driven by proliferation of the microvasculature, although many experiments *in vitro* have been performed with human umbilical vein endothelial cells (HUVECs), which are macrovascular cells. Furthermore, the authors pointed out that human skin microvascular endothelial cells, unlike other endothelial cell types of humans as well as cats, produced VEGF upon infection with *B. henselae*, thus suggesting that additional autocrine signaling could play a role in vascular tumor formation *in vivo* and may be a reason why pathological angiogenesis as a symptom of *Bartonella* infection preferentially affects the skin. However, an earlier study reported that HMEC-1 cells, another human skin microvascular endothelial cell line (3), lacked VEGF secretion in response to *B. henselae* infection (355).

Since endothelial cells infected with *B. henselae* typically do not secrete VEGF, a key role for VEGF production in other cell types and paracrine signaling to endothelial cells in the vasoproliferative tumor seems very likely, especially when considering the histological findings for verruga peruana nodules (see above). It has in fact been demonstrated that culture supernatants of human cells infected with *B. henselae* (Ea.hy 926 cells [225] and THP-1 macrophages [355]) could stimulate the growth of endothelial cells, undoubtedly because they contained VEGF and possibly other proangiogenic factors such as IL-1β (355) or IL-8 (284). Similar to the direct effects of *B. henselae* infection and indicative of mutual amplification, this paracrine stimulation was shown to trigger the secretion of various proangiogenic and antiapoptotic factors, particularly IL-8 or monocyte chemoattractant protein 1 (MCP-1) (225, 284). These cytokines are known for their chemotactic properties, and the culture supernatants of endothelial cells infected with *B. henselae* were indeed able to induce chemotaxis in THP-1 macrophages, thus indicating that bartonellae in the vascular tumors could promote the immigration of VEGF-secreting cells (282).

Taken together, these results imply a model for pathological angiogenesis in which proangiogenic effects of both direct stimulation by *B. henselae* infection and autocrine IL-8 signaling drive endothelial cell proliferation and inhibition of apoptosis together with paracrine signaling from infected nonendothelial cells such as the surrounding epithelium or infiltrating macrophages. Macrophages, as potent VEGF-secreting effector cells, could be attracted by chemokine secretion from the infected endothelium and build up a paracrine loop of mutual positive feedback that keeps up the complex angiogenic programs and finally results in vascular tumor formation (discussed in references 113 and 221).

Pathological angiogenesis due to infection with *B. henselae* has so far not been demonstrated in cats (61, 62) but was found in dogs, for example, upon infection with *B. henselae* or *B. vinsonii* subsp. *berkhoffii* (227, 441). However, it is reasonable to assume that the pathological angiogenesis observed in certain hosts and in response to certain *Bartonella* species (including *B. bacilliformis*) may reflect bacterial activities that evolved in the genus *Bartonella* for the colonization of the primary niche in the reservoir host, possibly for the maintenance of a locally restricted shelter in the microvasculature. Assuming that these activities would be potent but tightly balanced and regulated in order to keep up the stealth infection strategy, it is easy to imagine that differences in the responsiveness of cells in distinct hosts may cause an imbalance and finally trigger escalating feedback loops that would drive vascular tumor formation around the bacteria.

Infection of Erythrocytes

After colonization of the primary niche during *Bartonella* infection, the bacteria are seeded into the bloodstream, where they infect erythrocytes, thus establishing a protected niche that is competent for vector transmission in order to proceed in the infection cycle (89).

Entry in three steps: adhesion, deformation, and invasion. Most experiments investigating the phenomenology of erythrocyte infection *in vitro* have been performed with *B. bacilliformis*, but the occasional use of *B. henselae* as a modern species and the wide conservation of most bacterial factors suggest that the overall process is probably similar among the bartonellae (see below). However, particular disparities in the arsenal of dedicated virulence factors and the comparison of *in vivo* infection models using modern species (e.g., *B. tribocorum* [394] or *B. henselae* [1]) to the pathology of *B. bacilliformis* made it obvious that prominent differences must exist at least between *B. bacilliformis* and the other bartonellae as well as between flagellated and nonmotile species (see below).

In general, the seeding of *Bartonella* into the circulation seems to be followed by a sequence of adhesion, deformation, invasion, and finally intraerythrocytic persistence. Adhesion to erythrocytes as a first step of colonization was found to be a critical determinant of host specificity for the modern species (427) but apparently not for *B. bacilliformis*, since this species is known to adhere to cat erythrocytes (201), although it is restricted to infecting humans and closely related primates. Walker and Winkler reported that *B. bacilliformis* poorly adhered to human erythrocytes that had been treated with glucosidases, while the application of unspecific proteases (subtilisin or pronase) strongly favored adhesion (434). They thus concluded that *B. bacilliformis* might bind a glycolipid that would be uncovered from surface proteins by the proteases but might be destroyed by the glucosidases. Other studies focused on the interaction of *B. bacilliformis* with host proteins on erythrocyte membranes, although it is not clear which interaction partners might serve as receptors for adhesion and which ones might possibly be involved in the invasion process. A first report showed that the sets of erythrocyte proteins bound by *B. bacilliformis* and *B. henselae* are remarkably similar and clearly include actin and spectrin (201), while a second study provided solid evidence that *B. bacilliformis* interacts with the α and β subunits of spectrin, band 3 protein, glycophorin A, and monomeric as well as dimeric glycophorin B (63), thereby specifying the previous results. Binding of *B. bacilliformis* to glycophorin B from solubilized erythrocytes was considerably enhanced if these had been treated with trypsin or neuraminidase before, but chemical removal of the carbohydrate moieties from erythrocytes abolished binding to all proteins (63). These results indicate that the binding site of glycophorin B might be somehow masked under the conditions used and that binding of *B. bacilliformis* to erythrocyte proteins apparently involves their glycosyl chains. Interestingly, both trypsin and neuraminidase had only a slight, statistically insignificant effect on the adhesion of *B. bacilliformis* to whole erythrocytes, suggesting either that glycophorin B binding may not be critical for this process or that the bacterium could express a factor providing access to glycophorin B on red blood cells. Similarly, no significant effect of trypsin or neuraminidase treatment has been observed in an *in vitro* infection model with cat erythrocytes and *B. henselae*, but, just as for *B. bacilliformis* adhesion, the application of pronase led to an increased level of erythrocyte invasion (285).

Benson et al. showed that erythrocytes developed progressing indentations and invaginations at the sites where *B. bacilliformis* attached (31), a process that is known as deformation and was also confirmed for *B. henselae* (201). The molecular mechanism underlying deformation and the contribution of a dedicated, small molecule from *Bartonella* (see "Deformin" below) have not been resolved so far, but it seems as if the pits on the erythrocyte surface would provide entry sites for *Bartonella* (31).

Like that of deformation, the molecular mechanism of erythrocyte invasion is not well understood, but the interaction with band 3 protein, spectrins, and glycophorins seems conspicuous, since these proteins also serve as receptors for *Plasmodium falciparum*, the etiological agent of malaria (137, 158, 280, 418). Band 3 protein is part of a complex that connects the erythrocyte membrane to the underlying spectrin-actin cytoskeletal network (41, 209, 302), which is essential to maintain the shape and stability of red blood cells (for a recent review on erythrocyte membranes, see reference 303). Disconnecting this network from the membrane in order to locally weaken surface integrity (e.g., by degrading components like spectrin) is a common strategy of pathogenic parasites, including *Plasmodium* (112, 141, 171), and it seems reasonable to speculate that similar processes may also play a role at some stage of *Bartonella* infection. Importantly, mature erythrocytes are generally nonendocytic under physiological conditions and exhibit considerable resistance against the formation of endovesicles (discussed as a conceptual obstacle to *Plasmodium* infection in reference 308). However, the capacity of *B. tribocorum* to infect mature erythrocytes of its reservoir host has been demonstrated *in vivo*, and corresponding processes have repeatedly been observed *in vitro*, for example in an early study describing the phenomenology of *B. bacilliformis* invasion as "forced endocytosis" (31). A possible role for glycophorin B in the invasion process of *B. bacilliformis* could explain part of the host restriction of this species and cannot be directly conserved in many other bartonellae, since this protein evolved only in primates (352). In addition to direct interactions with the erythrocyte, bacterial motility was shown to be critical for the invasion process of *B. bacilliformis* and hence probably for the flagellated bartonellae in general (see below), potentially by providing mechanical force. It is apparent that erythrocyte invasion should be a relatively efficient process, since results with human serum and *B. henselae* revealed that the pathogen was efficiently killed by complement activation (362). Similarly, the preincubation of *B. bacilliformis* with different sera resulted in bacterial aggregates that were unable to deform or enter erythrocytes (287). Specific antibodies in the serum did not lead to increased killing of *B. henselae* but favored elimination of the bacteria by phagocytes (362), and antibodies against flagellin appear to inhibit erythrocyte invasion of the flagellated species (see "Flagella" below).

Intraerythrocytic persistence. It has not been resolved in great detail how erythrocyte infection proceeds after the invasion process, e.g., in what way the bacteria gain access to the nutrients inside the red blood cell. *B. bacilliformis* was reported to end up in intraerythrocytic vacuoles when the invaginations from membrane deformation bud off during "forced endocytosis." However, the bacteria also occasionally appeared in the lumen of infected erythrocytes *in vitro* (31), but it is not clear if the bartonellae are able to actively leave the vacuole under physiological conditions. The examination of rat erythrocytes from *in vivo* infection with *B. tribocorum* indicated that the bacteria remain inside a membrane-bound compartment (394).

Experiments on the rat model also indicated that red blood cells would be initially infected by not more than one or two bacteria, which divide two or three times, giving rise to eight bacteria per erythrocyte on average, and then persist for the residual life span of the red blood cell, which was apparently not shortened by infection (394). These results are supported by studies with other modern species reporting either five bacteria (*B. quintana* in human patients [367]) or only one bacterium (*B. henselae* in naturally infected cats [369]) per erythrocyte *in vivo*. Available data for *B. bacilliformis* confirm a similarly low ratio of ca. 4 bacteria per red blood cell (370). Interestingly, it seems as if not only the number of bacteria per infected erythrocyte but also the proportion of colonized red blood cells during *Bartonella* bacteremia would be rather low, at least when considering infections caused by the modern species. The proportion of infected erythrocytes ranges from well below 1% in the cases of *B. quintana* in humans (367) and *B. tribocorum* in rats (394) to about 5% in cats infected with *B. henselae* (234, 369). The course of bacteremia caused by the modern species in reservoir hosts has been investigated extensively using various experimental models as well as naturally infected animals, and the bacteremia was shown to persist usually subclinically for several months or even for considerably more than 1 year in both settings (see references 1, 235, 238, and 443; see also literature cited in "Host Specificity" above). Bacterial titers in the blood can reach 10^6 or even 10^7 (241, 443) but are usually two or more orders of magnitude lower (21, 45, 78, 88, 92), a variation possibly dependent on both the *Bartonella* and host species, immunological characteristics, and infection phase. The duration of *Bartonella* bacteremia in nature is not known (51) and is difficult to determine due to relapsing bacteremia from the primary niche and reinfections promoted by the high prevalence of *Bartonella* bacteremia among most communities of wild mammals (see examples in reference 211 and above).

In contrast to the modern species, *B. bacilliformis* can infect up to 100% of the host's erythrocytes (around 60% on average [271]) and thereby trigger a devastating hemolytic anemia during Oroya fever, the acute phase of Carrion's disease (272). The processes underlying the induction of hemolytic anemia by this pathogen are not well understood, but several studies have brought consensus that the red blood cells are not directly destroyed by a bacterial factor but rather by a host response (356, 359, 434). Based on both the examination of human patients and *in vitro* data, it is apparent that hemolytic anemia arises due to the elimination of many infected erythrocytes by mononuclear phagocytes, possibly upon recognition of abnormal surface properties. Such hemophagocytosis usually occurs in spleen, lymph nodes, and liver and leads to fever, hepatosplenomegaly, lymphadenopathy, and anemia (142, 208), which are also commonly observed during Oroya fever (272). Strikingly, Reynafarje and Ramos could show by the inspection of human patients suffering from Oroya fever that infected erythrocytes are preferentially destroyed in liver and spleen (356), and hemophagocytosis of red blood cells infected with *B. bacilliformis* was demonstrated histologically (359). Furthermore, the mechanical stability of erythrocytes in patients suffering from Oroya fever appears to be frequently decreased, which may also contribute to hemolysis (356).

Importantly, immune-mediated hemolytic anemia has also been reported as a rare complication of infections with modern bartonellae (50, 159, 424), implying that the bacterial factor(s) or strategy triggering hemophagocytosis may be conserved but somehow masked or more tightly regulated in these species. On the other hand, persistent and asymptomatic bacteremia of *B. bacilliformis* seems to be relatively frequent in the areas of endemicity (see above), indicating that the devastating hemolysis during Oroya fever may not be a necessary part of the infection strategy of this species.

Immune Evasion and Immunomodulation

Bartonella characteristically avoids elicitation of a host immune response by means of passive camouflage and active deception as part of its stealth infection strategy (outlined in reference 288). This bipartite strategy of "anti-immunology" (see reference 140 for a review) aims at colonization of the intraerythrocytic niche, where the bacteria are protected from both innate and adaptive immunity and persist in a compartment competent for vector transmission. In short, hiding and modification of pathogen-associated molecular patterns (PAMPs) like lipopolysaccharides (LPS) or flagella (see below) allow *Bartonella* to prevent its recognition as a bacterial pathogen. Additionally, *Bartonella* manipulates the host immune system on a systemic scale to achieve a state of immunological attenuation. Although most research has been performed on only a few species, available evidence allows the assumption that the general mechanisms of how *Bartonella* deals with the host immune system might be similar throughout the genus. However, it is clear that certain details of immunopathology might differ from species to species due to peculiarities in the particular process of host adaptation. The examples of passive immune evasion during *Bartonella* infections are discussed together with the corresponding bacterial factors at a later point in this review, so we focus here on how *Bartonella* actively obstructs functions of the host's immune system and how the immune system may control or eradicate the pathogen.

Current knowledge strongly suggests that the stimulation of IL-10 secretion is an important part of the immune modulation by *Bartonella*. This cytokine is a key regulator of immunity and has a moderating effect on immune responses by suppressing functions of various immune cells, including T helper cells, monocytes/macrophages, and dendritic cells, thus interfering both with innate immunity and with the establishment of an adaptive immune response. Although the resulting low level of inflammation may counteract acute pathology in the host under some circumstances, it is obvious that the effects of elevated IL-10 secretion greatly favor an asymptomatic, persistent course of infection as it is advantageous for *Bartonella* (see reference 104 for a recent review on IL-10). It has been proposed that the IL-10 level during infections may pose a balance between consequences that are more in favor of the host (e.g., prevention of tissue damage) or the pathogen (e.g., dampening the immune system), and the stimulation of IL-10 production was proposed to be a common feature of many intracellular pathogens that establish persistent infections (106). The central role of IL-10 for *Bartonella* has been strikingly demonstrated in the murine model of *B. birtlesii*, since the pathogen was unable to establish bacteremia in IL-10 knockout mice (275). Consistent with this, humans infected with *B. quintana* showed elevated IL-10 levels and exhibited an "attenuated inflammatory profile" (72). *B. henselae* promoted the secretion of IL-10 in mice, cats, and humans *in vitro* or *in vivo* (215, 328, 431), although the additional pattern of cytokine secretion elicited in the different hosts was not consistent and may contribute to the distinct outcomes of infection in reservoir and incidental hosts.

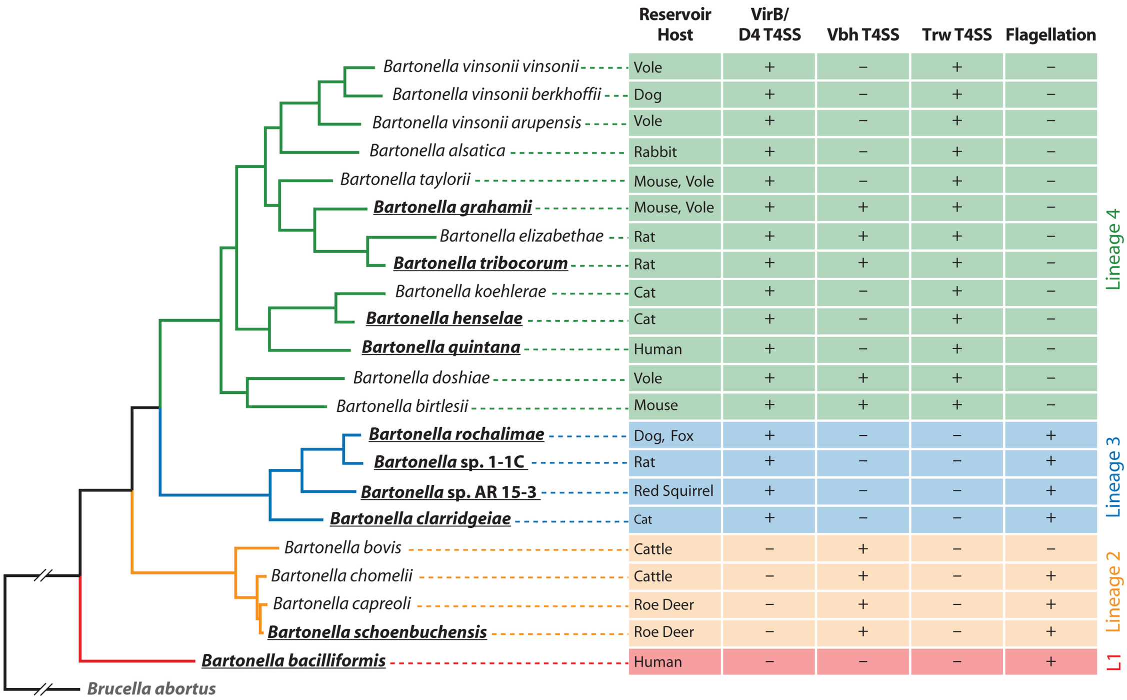
With respect to reservoir host infections, the study investigating mouse mutants in the *B. birtlesii* infection model further found that bacteremia in CD4 knockout mice (i.e., lacking T helper cells) was longer and featured higher bacterial titers than infections in wild-type mice (275). The authors concluded that this effect may derive from the key roles of humoral immunity as well as professional phagocytes in clearing *Bartonella* infection, both of which would require T helper cells (see reference 436 for a review). In contrast to the case for the CD4 knockout mice, infections in CD8 knockout mice were indistinguishable from those in wild-type animals. These results are complementary to those of another study using a mouse reservoir host model but infecting with *B. grahamii* (233). In this study, the lack of B cells as well as the lack of B and T cells considerably prolonged *Bartonella* bacteremia. Consistent with the findings previously mentioned, the authors proposed that mice lacking B cells would be unable to produce antibodies against *Bartonella* that could eliminate the bacteria seeded from the primary niche, similar to what was observed in the *B. tribocorum* rat infection model (394). Transferring immune serum from infected wild-type mice to B-cell-deficient mice was sufficient to restore a course of bacteremia congruent with the wild-type situation (233). A T_h2 response and corresponding activation of humoral immunity via IL-4 were also detected in cats naturally infected with *B. henselae* (212). The production of specific antibodies played a great part in removing *Bartonella* from the bloodstream but was apparently not suited to eliminate potentially intracellular bacteria from the primary niche, since relapsing bacteremia was observed. This finding is supported by another report showing that antibodies against *B. henselae* can prevent pathology in infected cats but fail to protect against the establishment of bacteremia (321). A further study proposed a central role for a T_h1 response involving the secretion of gamma interferon (IFN-γ) as well as tumor necrosis factor alpha (TNF-α) and stimulating cellular immunity in the eradication of *B. henselae* from experimentally infected cats (214). Studies investigating dogs infected with *B. vinsonii* subsp. *berkhoffii* revealed pleiotropic impairment of the host immune system coinciding with *Bartonella* bacteremia in the presence of considerable titers of specific antibodies (329, 330). The symptoms were deficiency in bacterial phagocytosis, an increase in major histocompatibility complex class II (MHC-II)-negative B cells (possibly due to hampered antigen presentation), an increase in naive $CD4^+$ T cells apparently deriving from abortive proliferation, and a decrease in $CD8^+$ T cells, which further seemed to be functionally handicapped. The effect of *Bartonella* infection on lymphocyte cell numbers appears to differ between the species, since cats infected with *B. henselae* were reported to exhibit a transient decrease in $CD4^+$ T-cell numbers (214), but the reason for or importance of these conflicting results is unclear. Taken together, instances of both cellular and humoral immunity seem to be necessary for complete eradication of *Bartonella* from reservoir hosts, and the pathogen apparently actively counteracts the establishment of such an immune response.

When comparing infections with modern *Bartonella* species in different hosts, a dichotomy between the usually self-limiting but possibly morbid course of infection in incidental hosts and the characteristic persistent but mostly asymptomatic infections in reservoir hosts is apparent. Various studies investigating infections of mice with *B. henselae* reported that the bacteria failed to cause bacteremia and elicited a T_h1 response with the secretion of IFN-γ that reliably eliminated the pathogen (16, 215, 219). The apparent importance of IFN-γ was interpreted to suggest a leading role for phagocytes and T-helper-mediated immunity in clearing *Bartonella* infections (220). These findings are supported by *in vitro* results showing that the activation of murine macrophages by IFN-γ prevented persistence of *B. henselae* in these cells and induced killing of intracellular bacteria, primarily by the production of nitric oxide (309). In humans, infections with *B. henselae* usually lead to cat scratch disease that elicits low to moderate morbidity and is typically self-limiting (see above). The characteristic swelling of lymph nodes derives from the formation of B-cell-rich granulomas that apparently feature continuous recruitment and stimulation of macrophages (397, 431). Similar lymphadenopathy could be observed in a mouse model for cat scratch disease, though no granuloma formation was evident and the swelling was mostly caused by the immigration and proliferation of B cells (244). Interestingly, the use of *B. grahamii* (infecting mice as its reservoir host) instead of *B. henselae* in the same study did not result in comparable lymphadenopathy unless the bacteria were sonicated before inoculation, though infecting IFN-α/β receptor-deficient mice yielded lymphadenopathy with both *Bartonella* species. These results suggest that the bartonellae may actively induce the secretion of IFN-α/β to counteract local immune responses via mechanisms that exhibit a certain host specificity.

In conclusion, these observations in mice and humans indicate that *Bartonella* immunomodulation may be impaired in incidental hosts and allow cellular immunity to clear the infection, especially since the inability to invade erythrocytes deprives the pathogen of its most important shelter.

The course of infection in general seems to critically depend on the ability of the host to launch an effective immune response against *Bartonella*: while most patients do not show signs of severe illness, cat scratch disease and other bartonelloses can develop into devastating, often systemic infections, particularly in immunocompromised individuals such as those suffering from AIDS but also in immunodeficient animals. It was thus proposed that an impairment of cellular immunity may enable *Bartonella* to colonize various sites of the mammal body due to its stealth properties (220). Reservoir host infections of the modern species seem to establish an antibody-mediated protective immunity that prevents reinfection once the bacteria have been eliminated (161, 444), but that apparently does not confer cross-protection against distinct bartonellae (442).

Although the molecular aspects of immune modulation during infections with *B. bacilliformis* have been scarcely investigated, a transient immunosuppression, including $CD4^+$ T-cell lymphopenia and an impairment of cellular immunity during Oroya fever, seems established in the field (150, 152, 191, 271) and is strikingly reminiscent of AIDS (417). Two studies confirmed increased levels of IL-10 in patients infected with *B. bacilliformis* (191, 192), strongly suggesting that enhanced secretion of this cytokine could participate in the immunosuppression as observed for the modern species. The most drastic difference in the immunopathology of *B. bacilliformis* compared to that of the modern species is that the majority of infected erythrocytes are usually destroyed by mononuclear phagocytes during Oroya fever, a condition rarely observed in infections with other bartonellae. Furthermore, the characteristic immunosuppression during Oroya fever makes the patients prone to secondary infections as a severe complication (see above). Secondary infections during infections with the modern *Bartonella* species have rarely been reported (412) and seem not to affect course or severity of infection (411), although coinfection with two or more different bartonellae is not uncommon (examples in references 54 and 336). Like for infections with the modern species, patients suffering from infections with *B. bacilliformis* seem to acquire antibody-mediated immunity against the pathogen, which is generally believed to abolish the acute phase of the disease. The considerable incidence of chronic carriers, the high seroprevalence of *B. bacilliformis*, and the relatively low rate of Oroya fever compared to verruga peruana among natives in the regions of endemicity seem to indicate that, like for the modern species, antibodies are not suited to eliminate the bacterium from



	Reservoir Host	VirB/ D4 T4SS	Vbh T4SS	Trw T4SS	Flagellation
Bartonella vinsonii vinsonii	Vole	+	–	+	–
Bartonella vinsonii berkhoffii	Dog	+	–	+	–
Bartonella vinsonii arupensis	Vole	+	–	+	–
Bartonella alsatica	Rabbit	+	–	+	–
Bartonella taylorii	Mouse, Vole	+	–	+	–
Bartonella grahamii	Mouse, Vole	+	+	+	–
Bartonella elizabethae	Rat	+	+	+	–
Bartonella tribocorum	Rat	+	+	+	–
Bartonella koehlerae	Cat	+	–	+	–
Bartonella henselae	Cat	+	–	+	–
Bartonella quintana	Human	+	–	+	–
Bartonella doshiae	Vole	+	+	+	–
Bartonella birtlesii	Mouse	+	+	+	–
Bartonella rochalimae	Dog, Fox	+	–	–	+
***Bartonella* sp. 1-1C**	Rat	+	–	–	+
***Bartonella* sp. AR 15-3**	Red Squirrel	+	–	–	+
Bartonella clarridgeiae	Cat	+	–	–	+
Bartonella bovis	Cattle	–	+	–	–
Bartonella chomelii	Cattle	–	+	–	+
Bartonella capreoli	Roe Deer	–	+	–	+
Bartonella schoenbuchensis	Roe Deer	–	+	–	+
Bartonella bacilliformis	Human	–	–	–	+

FIG 2 Phylogenetic structure and distribution of key virulence factors within *Bartonella*. The phylogenetic tree is based on a maximum-likelihood analysis of close to 500 genes of the 10 sequenced *Bartonella* species (bold and underlined) and *Brucella abortus*. Additional species were included using the sequences of a set of housekeeping genes (for details, see reference 134). The separation of the genus *Bartonella* into the three modern lineages 2 to 4 and the ancestral *B. bacilliformis* (lineage 1) is striking. Furthermore, type IV secretion systems are present in every modern species but not in *B. bacilliformis*, and flagellation as an ancient virulence determinant has apparently been replaced by the Trw T4SS in lineage 4. (Adapted from reference 134 with permission.)

niches outside the blood circulation and that maternal protection may exist (discussed in references 180, 218, and 220).

EVOLUTION OF THE GENUS *BARTONELLA*

The entry of *Bartonella* into the postgenomic era and the associated quantum leap in our understanding of *Bartonella* evolution has generated valuable knowledge not only for molecular microbiology but also for clinical science. Not only did it contribute to our awareness of the wide array of *Bartonella* virulence factors by enabling signature-tagged mutagenesis (STM) screens (373), but in addition it allowed us to get an idea of the evolutionary mechanisms underlying the remarkable host adaptability of this genus, which is of obvious interest in the context of its zoonotic potential. The complete genome sequences of 10 *Bartonella* species of all four phylogenetic lineages (see below) are available today and thus provide a broad basis for comparative analysis (134).

As a first result, genomic and phylogenetic analyses confirmed the disparity between *B. bacilliformis* as a deadly pathogen and the modern bartonellae, which exhibit a refined infection strategy that includes diminished morbidity and mortality. It was shown that *B. bacilliformis* is isolated within the *Bartonella* phylogeny as the only known representative of an ancient, deep-branching lineage (called lineage 1), while the other, modern bartonellae form three phylogenetic clusters of rather closely related species (lineages 2 to 4) (Fig. 2). These clusters apparently evolved rather recently via at least two independent adaptive radiations and thus contain groups of species that are adapted to infecting various mammalian reservoir hosts (134, 374). While the species of lineage 2 (e.g., *B. schoenbuchensis*) are still characterized by a certain limited host spectrum and have been isolated from different ruminants, the species of lineage 3 (e.g., *B. clarridgeiae*) and lineage 4 (e.g., *B. henselae*) infect diverse mammals as their reservoir hosts (134).

Genomic and pathogenomic analyses of the *Bartonella* genomes revealed that the different species share a core genome whose characteristics reflect the adaptation to a host-associated lifestyle, e.g., by a certain degree of genome reduction, although the genomes of some species have also been inflated, particularly by the acquisition of various phage-related elements (reviewed in reference 133). One striking example of a specific adaptation to the hemotropic lifestyle of the bartonellae is their apparent inability to synthesize heme (6), thus explaining why these bacteria are critically dependent on the availability of this compound that is usually abundant in the niches they colonize (see, e.g., reference 377). In addition, it has been shown that *Bartonella* (*B. henselae*) uses amino acids and not sugars such as glucose as its primary carbon source, probably because amino acids are more easily accessible in the host, for example, via extracellular proteolysis (85). Analyses on the level of *Bartonella* strains and populations revealed that the bartonellae seem to evolve in clonal complexes that exhibit considerable genome dynamics, particularly by excision and insertion of genomic islands, loss and acquisition of plasmids, and both intraspecies and interspecies horizontal gene transfer (32, 33, 133, 197, 335).

Genomic analyses also revealed the presence of type IV secretion systems in the modern species as the most obvious difference from *B. bacilliformis* and thus proposed that these systems may have served as host adaptability factors responsible for the adaptive radiations of the modern lineages (374). The current model suggests that the acquisition of (probably conjugative) type IV secretion systems via horizontal gene transfer would have provided the raw material for the evolution of virulence factors that allowed common ancestors of the modern lineages to greatly refine their capabilities of host interaction. As a consequence, the emergence of such molecular tools apparently induced subtle changes in the common infection strategy of the bartonellae and thereby promoted elevated host adaptability and decreased morbidity as evolutionary key innovations that triggered the adaptive radiations (134, 374). The exact nature of this modulation of the common *Bartonella* infection strategy is not known, but it must affect one or more critical points of host interaction since functional inactivation of a type IV secretion system in different modern species has repeatedly resulted in avirulent strains (374, 427), although the *Bartonella* infection strategy had initially evolved in the absence of type IV secretion systems (115).

It is well known that *Bartonella* reservoir host infections characteristically feature more or less asymptomatic bacteremia but that infections in incidental hosts may manifest in certain morbidity. In conclusion, it seems reasonable to assume that those bartonellae causing discernible disease in their reservoir host may have emerged from a rather recent host switch and would not yet be fully adapted to the new reservoir host. Consistent with this, a recent evolutionary study on *Bartonella* proposed that incidental host infections may pose an appealing opportunity for a host shift (134), particularly since these infections can also be long lasting. It is apparent that *B. quintana*, the only modern species that infects humans as its reservoir host, is such a recently emerged species that evolved from *B. henselae*, and a comparative genomic analysis of both species concluded that they must have diverged less than 100,000 years ago (6). In addition, it has been repeatedly noted that *B. vinsonii* subsp. *berkhoffii* is a frequent cause of significant morbidity in its canine reservoir host and elicits symptoms that appear to be very similar to those observed during human infections with *B. quintana* or, particularly in immunocompromised patients, *B. henselae*. Infections with *B. vinsonii* subsp. *berkhoffii* in dogs were described to involve well-known conditions such as fever, lymphadenopathy, pathological angiogenesis, and endocarditis (49, 50, 52, 98, 441) and have thus even been proposed to serve as a model for human disease (330). It is apparent from the phylogeny of the bartonellae that the subcluster containing *B. vinsonii* subsp. *berkhoffii* exclusively contains rodent-infecting species such as the other subspecies of *B. vinsonii* or *B. taylorii*, while *B. koehlerae* and *B. henselae*, as the closest relatives of *B. quintana*, infect cats as their reservoir host (Fig. 2). The molecular mechanism of these host switches has not been resolved in detail, but the outstanding importance of host shifts for the zoonotic potential of the bartonellae cannot be underestimated. A recent study identified the adhesion to erythrocytes via the Trw type IV secretion system (T4SS) as one key element of host specificity, since erythrocyte adhesion is the necessary first step to intraerythrocytic persistence and appeared to be strictly host specific (427). However, the authors demonstrated that, as the only known exception to this rule, *B. quintana* was able to adhere to erythrocytes of both humans and cats, indicating that a mutation within the diverse array of Trw-associated surface structures (see below) may have allowed the *B. henselae* clone ancestral to *B. quintana* to close the infection cycle in humans.

When discussing the evolution of *Bartonella*, it is important to mention that the role of a gene transfer agent, i.e., a bacteriophage-like vehicle of horizontal gene transfer (described and reviewed in references 250 and 408), has been appreciated only recently and must not be confused with the occurrence of real bacteriophages in some species of this genus (for example, prophage I in *B. grahamii* [34] or another phage of *B. tribocorum* [374]). Corresponding bacteriophage-like particles (BLPs) with an icosahedral head that apparently pack ~14-kb pieces of linear, double-stranded DNA in a semirandom fashion were discovered early on and found to be morphologically similar in many *Bartonella* species, including *B. bacilliformis*, *B. henselae*, and *B. quintana* (11, 23, 47, 269, 421). The different reports showed that these particles may be found with or without a tail and not only in the bacterial supernatant but also attached to the bartonellae or intracellular. Furthermore, the BLPs seem to contain an invariable set of three proteins of approximately 32 kDa, 34 kDa, and 36 kDa as well as other minor constituents (11). The 36-kDa component has been termed PapA (for particle-associated protein A) and was revealed to be a phage tail protein (12). Remarkably, an early study described that BLPs inside the cytoplasm of *B. bacilliformis* lacked a tail, while those attached to the surface apparently adhered to the bacterium via their tail, indicating that PapA could be inserted into the outer membrane and somehow polymerize on the phage (421). A smaller protein of ca. 32 kDa was described as Pap31 (for particle-associated protein of 31 kDa) and subsequently revealed to be HbpA, a well-known virulence-factor of *Bartonella* (see "Hemin binding proteins" below). A recent study indicated that this protein might end up in the BLPs by coincidence because it is highly expressed under conditions of high hemin levels, and HbpA indeed was not detectable in BLPs of bacteria that had been cultured in the absence of hemin (34). Encouraged by the obvious relationship of the *Bartonella* BLPs to bacteriophages, extensive attempts to demonstrate a lytic release of these particles have been made, but bacterial lysis linked to BLPs could never be shown (11). However, the release of bacteriophage-like particles in liquid culture of *B. henselae* correlated with the entry of these bacteria into a post-exponential-phase "death phase" with a drop in bacterial viability and membrane integrity, but a causal relationship to the BLPs has not been established (85).

Although the semirandom nature of DNA incorporation into the BLPs of the gene transfer agent prompted other authors early on to propose these particles as a part or remnant of a mechanism for horizontal gene transfer (47), a conclusive model for such a process has been brought up only recently by Berglund et al. (34). Using *B. grahamii*, they could show that a highly variable region of the bacterial genome encoding various type V secretion systems as well as the VirB/D4 type IV secretion system was amplified by runoff replication and preferentially packed into the BLPs, thus providing at least one appealing mechanism that may account for the genomic diversity of the bartonellae in natural populations and the horizontal gene transfer of virulence factors.

VIRULENCE FACTORS

In order to draw a comprehensive picture of the molecular pathogenesis of *Bartonella* infections, we apply a broad definition of the term virulence factor and discuss bacterial factors that confer the "ability to enter, replicate and persist in a host" (105). We classify these factors in *Bartonella* according to the stage of infection at which their activity is mostly contributing to pathogenesis; i.e., we separate them depending on whether they are primarily involved in the interaction with nucleated host cells (see "The Primary Niche" below), contribute to blood-stage infection (see "Infection of Erythrocytes" below), or cannot be sorted into one of these categories (see "Other Virulence Factors" below).

Decades of studies, continuous advances in the molecular biology of *Bartonella*, and the entry of this genus into the postgenomic era have greatly increased the number of bacterial factors that are assumed to participate in virulence, but comparably little is known about the molecular pathogenesis and the particular function of many of them (6, 119, 123, 267, 294). A comprehensive view of the molecular pathogenesis of *Bartonella* is further complicated by the fact that the two common lab strains of *B. henselae*, by far the most well-studied species, have different genotypic backgrounds and differ in their arsenals of virulence factors. Two subtypes of *B. henselae* (I and II) have been distinguished based on serotypic and genotypic characteristics, and the Houston-1 typing strain belongs to group I, while the Marseille strain belongs to group II (197, 198, 251). Importantly, a two-dimensional (2D) gel electrophoresis analysis revealed that 95% of the protein spots were identical in the Houston-1 and the Marseille strains (452), thus making it possible to imagine yet-unknown differences in the arsenal of virulence factors, especially since the genome sequence of the Marseille strain is not available. Strains of the two groups of *B. henselae* have been reported to exhibit certain differences in the interaction with endothelial cells (82), although these findings could not be confirmed by a more recent study (37). Furthermore, the two subtypes of *B. henselae* have also been suggested to differ in pathology (83) as well as in their association with feline or human infection (44). Most critically, the prevailing variant of the Marseille strain expresses a functional BadA trimeric autotransporter adhesin (TAA) but no VirB/D4 T4SS, while the situation is the opposite in the most common variant of the Houston-1 strain (382), although both factors are most likely essential for productive infection *in vivo*. The evaluation of the available literature further suggests that other variants of Houston-1 and, most likely, the original isolate exhibited surface expression of BadA (for example, "well piliated" Houston-1 in reference 245), while the variant used for genome sequencing did not (361).

The Primary Niche

TAAs. Trimeric autotransporter adhesins (TAAs) are well-known virulence factors of Gram-negative bacteria and typically contribute to pathogenesis by binding to host proteins on cell surfaces, in the extracellular matrix (ECM), or as circulating factors (recently reviewed in reference 260). They form extracellular filaments composed of head and stalk domains that are assembled on a C-terminal membrane anchor, thus resulting in a characteristic "lollipop-like" structural architecture (103, 185, 260, 322). The membrane anchor inserts into the outer membrane as a trimeric β-barrel and allows passage followed by trimerization of the exported filament. Unlike the case for classical (monomeric) autotransporters, the trimerization of TAAs is crucial for their folding, stability, and activity (102). While the head of TAAs usually constitutes the "business end," the stalk serves primarily as an extender that presents the head to its host binding partners, although it may also directly contribute to the interaction (260, 363). The head and stalk are built up from a repetitive array of a limited set of domain modules, hence favoring events of sequence reshuffling and recombination that can alter the length of the stalk by repeat contraction or expansion and modulate binding specificities of the head (260, 286).

TAAs play important roles in the infection strategies of various pathogens such as *Yersinia* (YadA) (132), *Haemophilus* (Hia) (247), or *Neisseria* (NadA) (71). They can be found in the genomes of all *Bartonella* species sequenced so far, usually featuring several paralogous copies and pseudogenes (34, 157, 216, 450), and related proteins are encoded in the genomes of other *Rhizobiales* such as *Brucella* (e.g., BruAb1_0072 in *B. abortus* [170]). It has been shown in experimental infections with *B. birtlesii*, *B. tribocorum*, and *B. quintana* that TAAs of *Bartonella* are important virulence factors and necessary for successful colonization of the primary niche (267, 374, 427).

(i) BadA, a trimeric autotransporter adhesin of *Bartonella henselae*. Most research on the TAAs of *Bartonella* has been performed on *Bartonella* adhesin A (BadA) of *B. henselae*, a giant protein composed of more than 3,000 amino acids per polypeptide chain. BadA trimers form hair-like filaments of ~240 nm length on the bacterial surface (306) (Fig. 3), and their appearance was initially misinterpreted as being related to type IV pili (24). The key role of BadA in the molecular pathogenesis of *B. henselae* has been thoroughly studied with the Marseille strain (i.e., in the absence of a functional VirB/D4 T4SS), which exhibits a plethora of BadA-dependent phenotypes: bacterial autoagglutination, adhesion to host cells as well as extracellular matrix (ECM), inhibition of phagocytosis, and induction of a proangiogenic transcriptional program in target cells (360) (Fig. 3). As expected, the head of BadA was found to be sufficient for most of these phenotypes (217). Structural analysis of the head revealed that it is composed of three subdomains featuring an N-terminal domain similar to the head repeats of *Yersinia* YadA as well as a Trp ring domain and a GIN domain that show remarkable structural similarity to elements present in the head of *Haemophilus* Hia, but the assignment of particular functions to these head subdomains has remained enigmatic (413).

Solid experimental evidence indicates that BadA-mediated adhesion to endothelial cells may target β1-integrins and involve bridging via ECM proteins such as collagens or fibronectin (217, 360). Consistent with this, it was shown that *B. henselae* binds these components of the ECM and several others, like hyaluronate, laminin, and vitronectin, in a BadA-dependent manner (306, 360). Although the head of BadA was sufficient to bind endothelial cells as well as collagens, the stalk was required to bind fibronectin (217), and a recent study investigating the effect of dynamic flow conditions revealed that BadA was particularly important for endothelial cell attachment in the presence of shear stress as expected *in vivo* (306).

In addition to host cell adhesion, the head of BadA was also found to be sufficient as a trigger for the proangiogenic transcriptional program that the Marseille strain of *B. henselae* induces in host cells (217). A study by Kempf et al. indicated that this reprogramming of gene expression is based primarily on the activation of NF-κB as well as hypoxia-inducible factor 1 (HIF-1) and involves the upregulation of 20 genes in infected HeLa cells (222). Similar to the well-known modulation of NF-κB by bacterial pathogens (349), HIF-1 activation has recently been recognized as a common theme in human infections (438) and is a key trigger of various angiogenic processes (344). Consistent with this, the majority of genes upregulated in HeLa cells upon infection with *B. henselae* Marseille could be linked to angiogenesis, cell metabolism, or growth and included genes for potent proangiogenic factors such as IL-8 (regulated by NF-κB) and VEGF (regulated by

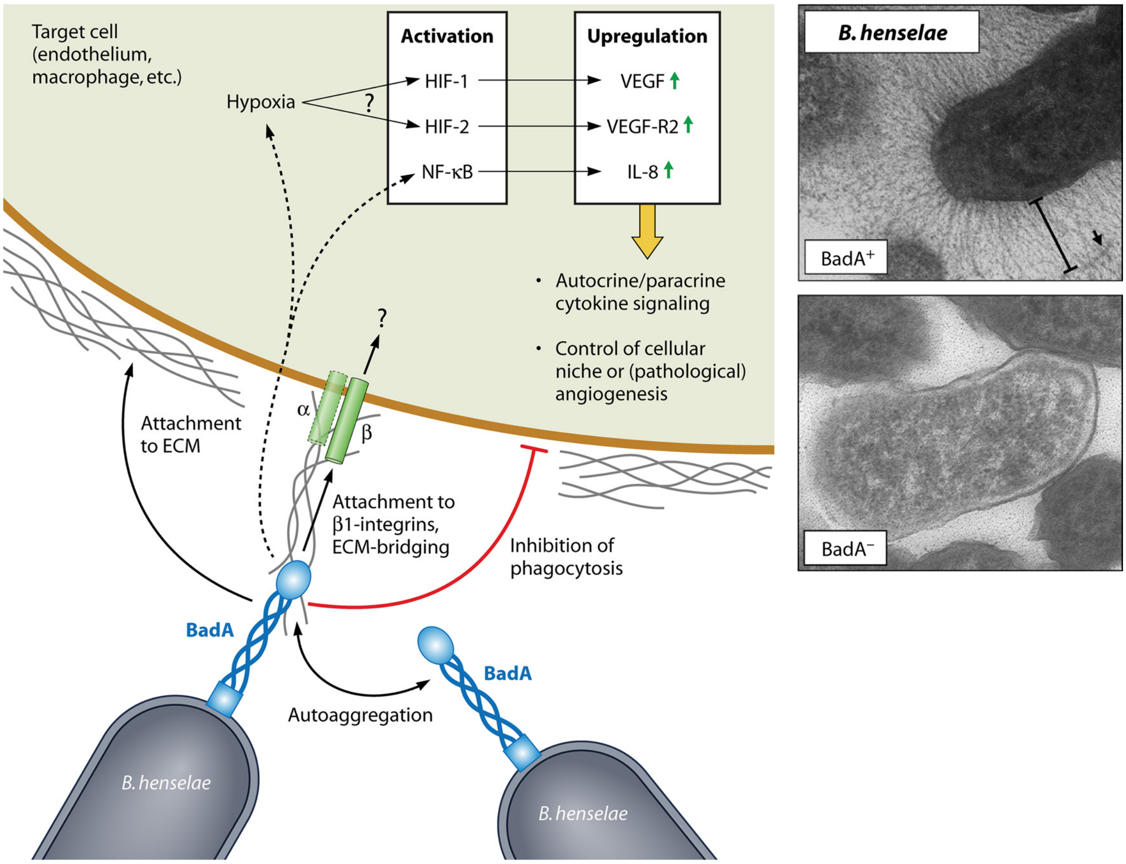


FIG 3 BadA, a trimeric autotransporter adhesin of *B. henselae*. Left, the different virulence phenotypes that have been attributed to BadA in experiments with the *B. henselae* Marseille strain are summarized in a schematic drawing. Note that a direct or indirect causal relationship between the attachment to β1-integrins and the activation of HIF-1/2 or NF-κB seems likely but has not been demonstrated. Right, transmission electron micrographs of wild-type *B. henselae* Marseille and a mutant lacking BadA expression. Not only is the remarkable size of the trimeric autotransporter adhesin obvious (bar, ca. 240 nm), but a close view also reveals spots of electron density that may be the head domains (arrow). (Right panels adapted from reference 306 with permission.)

HIF-1) (222). The activation of both NF-κB and HIF-1 was found to be dependent on BadA (217) but seemed to proceed independently (222), although considerable cross talk and interdependence between the NF-κB and HIF-1 pathways have been described in the literature (415, 426). Interestingly, the infection of HUVECs instead of HeLa cells resulted in the upregulation of more genes of the NF-κB regulon (e.g., that for MCP-1) and activated HIF-2 in addition to HIF-1, thus stimulating the expression of VEGF-R2. In line with the great majority of reports for *Bartonella*, both cell types secreted IL-8, while considerable VEGF secretion was detected only for HeLa cells, although corresponding transcriptional upregulation was observed in both cell types (222). In conclusion, it is apparent that the altered pattern of gene expression in response to BadA is part of the contact-dependent antiapoptotic and mitogenic effects of *B. henselae* (see above).

The molecular mechanism of how BadA mediates the activation of NF-κB and HIF-1/2 in host cells is not known, but Kempf et al. detected BadA-dependent hypoxia in infected cells that could trigger HIF-1/2 activation (222). Hypoxia was also shown to be a possible trigger of NF-κB activation (320), but it may also be induced by the interaction of BadA with β1-integrins via bridging of ECM proteins such as fibronectin. β1-Integrins are key players in angiogenesis (reviewed in reference 17), and the binding of fibronectin to $\alpha 5\beta 1$-integrins was shown to induce expression of proangiogenic genes, including that for IL-8, in endothelial cells via an NF-κB dependent pathway (228). It is therefore conceivable that such an interaction of BadA would induce the NF-κB part of the proangiogenic transcriptional program, especially since other TAAs such as *Yersinia* YadA or NadA of *Neisseria* were also shown to bind β1-integrins and to trigger the expression of IL-8 in host cells (130, 147, 310, 386).

The multiple roles of BadA during infections with *B. henselae* have not been fully resolved, but the activation of HIF-1 was detected by immunohistochemistry in monocytic phagocytes that infiltrated bacillary angiomatosis lesions in human patients (222). This finding supports the hypothesis that *Bartonella* would trigger the secretion of proangiogenic cytokines from nonendothelial cells like these phagocytes as part of a paracrine loop that drives pathological angiogenesis (222) (see above), especially since BadA was also shown to protect *B. henselae* from phagocytosis by macrophages via an unknown mechanism (360).

Despite such detailed knowledge on the role of BadA as a virulence factor of *B. henselae*, a paralogous TAA encoded close by has been described only recently and appears to be intact both in the Marseille strain and the Houston-1 strain (216). However, no experimental evidence on any kind of functionality of this protein has been published so far, but it was found to contain several Trp rings and GIN domains that are not present in some TAAs of other *Bartonella* species (322).

(ii) Trimeric autotransporter adhesins of other *Bartonella* species. Trimeric autotransporter adhesins can be found in every *Bartonella* species sequenced so far, but they vary considerably in length and show differences in their domain composition (recently reviewed in reference 322). Most research on the TAAs of *Bartonella* apart from BadA has been performed on the *B. quintana* homologs called Vomps (for "variably expressed outer membrane proteins") (450). The Vomp genes form a genomic locus of four genes (encoding VompA to -D), but frequent events of recombination between these genes apparently led to the inactivation of one or more of them in the different strains of *B. quintana*. Consistent with this, the deletion of several (but never all) Vomp genes was occasionally observed in strains isolated from experimentally infected rhesus macaques as well as from human patients. This phenomenon is most likely attributable to functional redundancy in combination with selective pressure of the host's immune system (450), since the Vomps are known to be immunodominant in human infection (43). However, a complete deletion of the Vomp locus resulted in an avirulent strain (267), consistent with the phenotype of TAA-deficient mutants in STM screens of *B. birtlesii* and *B. tribocorum* (374, 427). Regarding sequence diversity and similarity, VompA to -C appear to be very close and to lack the Trp ring domain and the GIN domain found in the BadA head, thus featuring only the part that is homologous to the YadA head repeats. In contrast to these three proteins, VompD also contains Trp ring and GIN domains (322, 413). Furthermore, the Vomps are much shorter than BadA (approximately 40 nm, compared to about 240 nm for *B. henselae* Marseille BadA [306]), which is mostly due to a considerably lower number of stalk repeats (322).

The biological role of the Vomps has been investigated in several studies, and they seem to play a role in adhesion and angiogenic reprogramming similar to that of *B. henselae* BadA. However, despite the presence of all four Vomp genes in the JK-31 strain of *B. quintana*, which is used as Vomp-positive wild-type strain, VompD seems not to be expressed in this strain (395), and thus no direct evidence for the function of this protein is available. A phenotypic comparison of the JK-31 strain with other strains lacking Vomp expression revealed that VompA to -C are apparently necessary for the induction of VEGF secretion in HeLa cells and THP-1 macrophages, but no significant contribution to attachment to these cells could be detected (395). Furthermore, VompC was found to bind collagen IV, while VompA was both necessary and sufficient for bacterial autoaggregation (450). However, a more recent study using the same strain revealed clear Vomp-dependent adhesion to endothelial cells under both static and dynamic flow conditions, and the authors speculated that this apparent discrepancy may be caused by the expression of a specific binding partner on endothelial cells but not on the other cell types (306). They further detected Vomp-dependent adherence of *B. quintana* to fibronectin (in contrast to a previous study [395]) and other ECM proteins such as collagens or laminins under static conditions, but this binding seemed to be rather unspecific. Under flow conditions, binding to most ECM components, except hyaluronate and vitronectin, was greatly decreased, indicating that these factors may be relevant targets of Vomp-mediated adhesion during human infection (306). In addition to obvious functions during infection of the mammal host, one or more of the Vomps may also play a role in transmission via the arthropod vector, for example, by contributing to adhesion in the flea gut (450).

The TAAs of other *Bartonella* species are less well characterized, but it appears as if one of the three homologs in *B. bacilliformis* contains a head similar to the shorter paralog of BadA, featuring several Trp rings and GIN domains, while the others seem more closely related to VompA to -C and lack these head subdomains (216). *B. tribocorum* encodes only one TAA, but this protein was described to be the longest TAA in *Bartonella* and features a BadA-like head with a Trp ring and GIN domain (322). The three TAAs of *B. vinsonii* subsp. *arupensis* (called BrpA to -C for *Bartonella* repeat protein) contain excessive repeats and were discovered in a study investigating *Bartonella* antigens because at least one of them, BrpA, seems to be immunodominant in a mouse infection model (157).

VirB-like T4SS. (i) The VirB/D4 T4SS and *Bartonella* effector proteins. Type IV secretion systems (T4SS) are macromolecular machineries prevalent among most groups of prokaryotes and originally mediated conjugation, i.e., the interbacterial transfer of genetic material (usually plasmids) in form of a nucleoprotein complex (7). Conjugation is a major mechanism of lateral gene transfer in bacteria and plays prominent roles in the dissemination of genes involved in virulence, antibiotic resistance, or other fitness traits among bacterial populations (317). Conjugative T4SS have repeatedly evolved into host-interacting machineries that are well known as bacterial virulence factors (146), typically contributing to pathogenesis by translocating effector proteins into eukaryotic target cells (19). Based on the nomenclature of the prototypic VirB/D4 T4SS of *Agrobacterium tumefaciens*, the type IV secretion (T4S) machinery consists of 10 essential components, VirB2 to -11, plus the type IV secretion coupling protein that serves as a substrate recognition module. The T4S machinery is assumed to assemble a translocation channel that spans both the inner and outer membranes and then merges into a surface filament. This filament mediates initial attachment to target cells and is thus called "sex pilus" in the context of conjugation. However, the precise mechanism of substrate translocation by T4SS remains poorly understood, although two major models propose either a piston mechanism or export through a pilus channel (77, 95, 435).

The VirB/D4 T4SS of *Bartonella* constitutes one of the most prominent virulence factors of this genus (115) and is present in all species of lineages 3 and 4 (134). It was discovered during the investigation of a 17-kDa antigen of *Bartonella henselae* that turned out to be a VirB5 homolog and encoded as part of a *virB2-virB11* operon (326, 387, 410). The two STM screens in *B. birtlesii* and *B. tribocorum* revealed that a functional VirB/D4 T4SS is essential for the pathogenicity of these species, and a segregation analysis with two T4SS complementation plasmids in the *B. tribocorum* infection model strongly indicated that it is required during colonization of the primary niche rather than for blood-stage infection (374, 392, 427). These results are further supported by *in vitro* infections with the *B. henselae* Houston-1 strain, which elicits various VirB/D4-dependent phenotypes in human endothelial cells (385). Taking the findings together, it is apparent that the VirB/D4 T4SS plays a major role in the manipulation of nucleated host cells during infections with a large group of modern *Bartonella* species.

(a) Bartonella effector proteins. The VirB/D4 T4SS contributes to *Bartonella* virulence by translocating Beps (*Bartonella* effector proteins) into host cells, where they subvert cellular functions in

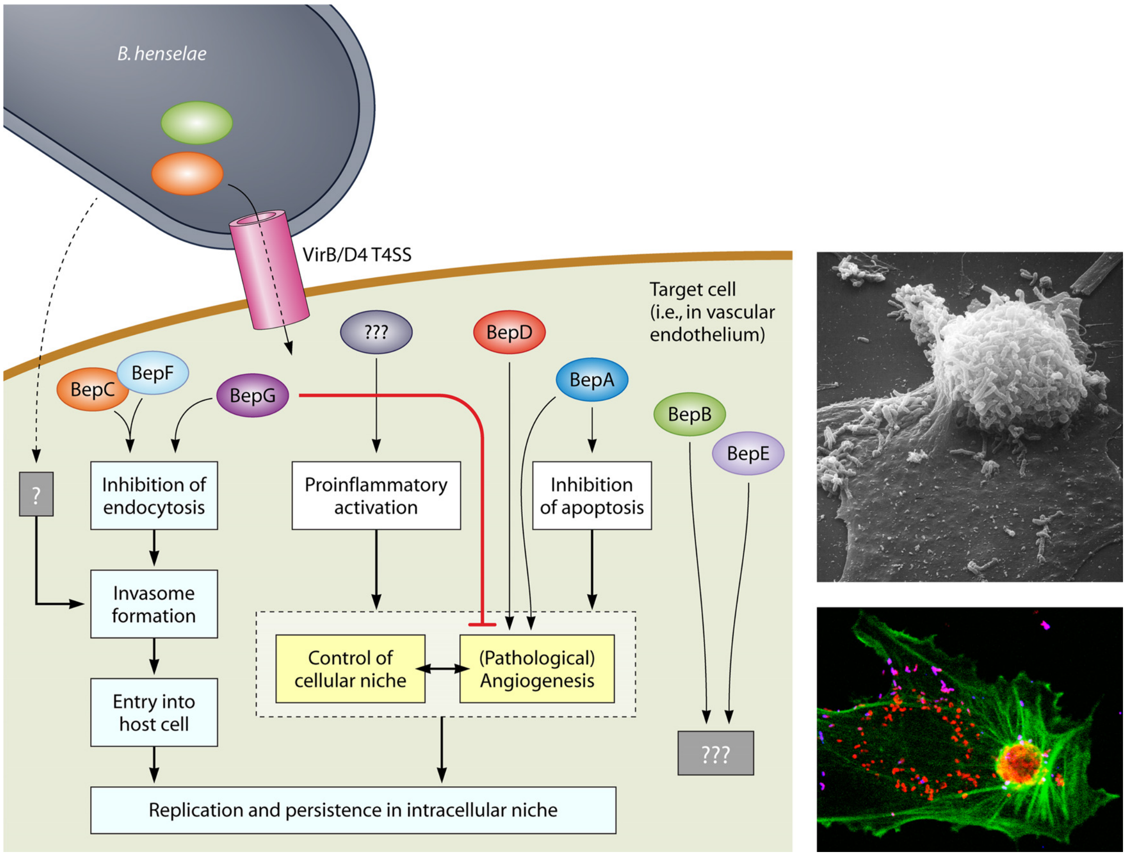


FIG 4 VirB/D4 T4SS and Beps of *B. henselae*. Left, the different virulence phenotypes of the VirB/D4 T4SS and the Beps are summarized in a schematic drawing. It becomes apparent that the diverse cellular activities converge toward the establishment, maintenance, and control of an intracellular niche. Right, the formation of an invasome on human endothelial cells infected with *B. henselae* is shown as visualized by different techniques (top, scanning electron microscopy; bottom, confocal microscopy and immunocytochemistry). In the bottom part intracellular bacteria appear red, extracellular bacteria appear purple, and the actin cytoskeleton is stained green. Note the remarkable actin ring structure forming around the bacterial aggregate. (Right panels adapted from reference 120 with permission.)

favor of the pathogen (Fig. 4). All known VirB/D4-dependent cellular phenotypes of *B. henselae* in the *in vitro* infection model are dependent on the delivery of at least one effector (385, 393). The Beps display a modular domain architecture with N-terminal host-interacting modules fused to a C-terminal secretion signal. This signal consists of a Bep intracellular delivery (BID) domain followed by a nonconserved tail sequence displaying a net positive charge as the only discernible feature (393).

The Beps of lineage 3 (e.g., *B. clarridgeiae*) and lineage 4 (e.g., *B. henselae*) can be sorted into 10 and 7 phylogenetic clades (i.e., orthologous groups), respectively. Most of these carry an N-terminal FIC (filamentation induced by cyclic AMP [cAMP]) domain that is usually followed by a small oligonucleotide/oligosaccharide binding (OB) fold and the C-terminal secretion signal (134). However, a subset of effectors in both lineage 3 and lineage 4 harbor other functional modules instead of FIC domains and feature either additional BID domains or tyrosine-containing motifs that are phosphorylated upon translocation into the host cell (374). Three of the seven Beps of *Bartonella henselae* (BepA to -C) display the FIC-OB-BID architecture, and four (BepD to -G) harbor other elements at their N termini, whereas in lineage 3, 9 of the 10 phylogenetic clades of Beps contain a FIC domain and only one bears tyrosine phosphorylation motifs instead (134).

Although various cellular phenotypes associated with single effectors or a subset of Beps have been reported (see below), comparatively little is known about interacting host factors or underlying molecular mechanisms. FIC domains as the putative host-interacting part of many *Bartonella* effector proteins are known to mediate AMPylation, i.e., the covalent transfer of an AMP moiety onto hydroxyl side chains of target proteins. This peculiar enzymatic activity has been examined using several virulence-associated proteins of other bacterial pathogens and was even detected for BepA of *B. henselae* (327, 371). However, no FIC domain of any *Bartonella* effector has so far been demonstrated to contribute to *Bartonella* virulence *in vitro* or *in vivo*.

Tyrosine phosphorylation motifs are a prevalent feature among bacterial effector proteins and usually promote infection by subverting host signaling cascades upon phosphorylation in the target cell (20). Consistent with this, Beps with tyrosine motifs from both lineage 3 and lineage 4 were shown to become phosphorylated in human cells (134, 393). Although their particular mode of action remains largely unknown, the phosphotyrosine motifs of BepD to -F were found to bind various SH2 domain-containing host proteins (400) that are key elements of cellular signaling (334). No phenotype during *in vitro* infection, however, has yet been demonstrated to depend on the tyrosine phosphorylation motifs of any *Bartonella* effector protein.

In contrast to the FIC domains and tyrosine phosphorylation motifs of the *Bartonella* effectors, the BID domain of *B. henselae* BepA was found to be sufficient for elicitation of the strong antiapoptotic and proangiogenic phenotype that is the hallmark of this effector protein (381, 384) (see below). Because the same domain also mediates translocation of BepA, it is apparent that BID domains of *Bartonella* effector proteins can have dual functions and act in host interaction and as signals for the type IV machinery. Conspicuously, various effector proteins contain several BID domains, although, for example, in the case of *B. henselae* BepG the C-terminal one alone is sufficient for translocation (358). It is hence more than likely that direct functions of BID domains in the manipulation of host cells are more widespread among *Bartonella* effectors, further highlighting the evolutionary versatility of these fascinating proteins.

(b) Evolution. It is well established that the VirB/D4 T4SS played a major role in the evolution of the modern bartonellae, but its own evolution also constitutes a fascinating matter of research. Generally, the work by Frank et al. showed that protein-secreting type IV secretion systems evolved out of conjugative ancestors various times independently (146), and it is hence not surprising that the closest known relatives of the VirB/D4 T4SS outside *Bartonella* are conjugative T4SS encoded on plasmids found in other *Rhizobiales* (374). Remarkably, the relaxase secretion signal of one of these, the AvhB T4SS on the pATC58 plasmid of *A. tumefaciens*, was sufficient to direct VirB/D4-dependent translocation of fusion proteins from *B. henselae* into human endothelial cells (393), and another study found that the VirB/D4 T4SS of *B. henselae* could transfer a relaxase loaded with plasmid DNA into human cells (391). These results suggest that the bipartite secretion signal of *Bartonella* effector proteins (BID domain plus positively charged tail) may have evolved in relaxases of alphaproteobacterial conjugation systems and was then simply adopted during the evolution of *Bartonella* effector proteins to deliver host-interacting modules into target cells (393).

The two separate arrays of *Bartonella* effector proteins in lineages 3 and 4 evolved convergently from one common ancestor, suggesting that a primordial VirB/D4 T4SS would have initially operated with a single effector protein. Since FIC domain-containing effectors are closest to the root of the *Bartonella* effector phylogeny in both lineages (BepA and Bep1, respectively), it is likely that the ancestral effector harbored a FIC domain and contributed to *Bartonella* pathogenesis via the AMPylation of host targets (134). The striking similarity of the VirB/D4 T4SS and the Vbh T4SS, a third and mostly plasmid-encoded type IV secretion system found in several *Bartonella* species, makes it likely that both systems might have diverged from a common ancestor rather recently in evolution (374). After chromosomal fixation of a primordial VirB/D4 T4SS in combination with an ancestral effector, successive rounds of gene duplication, diversification, and reshuffling generated the distinct effector arsenals of lineages 3 and 4. The diverse cellular phenotypes of the Beps encoded by *B. henselae* strongly suggest that their rapid evolution was driven by functional diversification with the acquisition of different roles in host interaction (358, 381, 384, 420). Although most clades of effector proteins still display the ancestral domain architecture, the N-terminal FIC domain has secondarily been replaced with other functional modules in effectors of both lineages independently. This remarkable parallel evolution generated novel types of effector proteins containing tandemly repeated tyrosine phosphorylation motifs and/or additional BID domains as presumable host-interacting elements (134).

(c) Cellular effects. The Houston-1 strain of *B. henselae* was found to elicit a wide array of VirB/Bep-dependent cellular phenotypes in an infection model with human endothelial cells that may correspond to the manipulation of nucleated host cells *in vivo*, e.g., during primary niche infection (115). These phenotypes comprise inhibition of apoptosis, activation of proinflammatory signaling via NF-κB, modulation of angiogenesis, and reorganization of the actin cytoskeleton during invasome formation (381, 385) (Fig. 4), but the molecular contribution of each single effector protein has been only partially resolved so far.

The entry of *B. henselae* Houston-1 into host cells via invasome formation proceeds in three steps and starts with bacterial adhesion and aggregation on the cell surface, accompanied by effector secretion. Subsequently, the bacterial aggregate is engulfed by cellular protrusions and finally internalized as a whole (358). The term "invasome" denotes the host cell structure that takes up the bacteria, comprising mostly stress fibers that form a compact F-actin ring underneath the bacterial aggregate (Fig. 4). Invasome formation is a slow process and takes at least 16 to 24 h, while the alternative pathway yielding BCVs takes only minutes or a few hours (120, 358). It was found that invasome formation is fully dependent on the translocation of *Bartonella* effector proteins and can be elicited by BepG alone or BepC in combination with BepF (358, 420). Surprisingly, these effectors apparently do not trigger invasome formation directly but seem rather to contribute indirectly by inhibiting endocytic uptake of *B. henselae.* The ectopic expression of BepG in human cells did not induce any discernible cytoskeletal rearrangements, although it was sufficient to restore invasome formation of an effectorless mutant of *B. henselae*. Furthermore, the expression of BepG, as well as BepC and BepF, interfered with endocytic uptake of inert microspheres (358, 420). Rhomberg et al. thus suggested that the inhibition of endocytosis could be a prerequisite that enables *B. henselae* to aggregate on the cell surface and thereby cluster host receptors bound to the bacteria. This clustering of cellular receptors (and not the direct activity of any *Bartonella* effector protein) may then trigger the massive actin rearrangements that lead to invasome formation. Consistent with this, the effectorless mutant of *B. henselae* Houston-1 enters human endothelial cells into BCVs (358). Recent work showed that *B. henselae* Houston-1 binds β1-integrins on host cells and activates them in a fibronectin-independent manner via talin-1 inside-out signaling, indicating that integrins may be a major receptor for *B. henselae* on host cells (419). This hypothesis is further supported by the finding that an extended, activated conformation of β1-integrins on human endothelial cells is necessary for effector translocation and is recruited to the sites of invasome formation. Moreover, the same study suggests that β1-integrin outside-in signaling via focal adhesion kinase (FAK) and Src to paxillin and vinculin may control the actin rearrangements that are the basis of invasome formation. These include both the reorganization of preexisting F-actin and the *de novo* polymerization via pathways involving Rac1/Scar1/WAVE/Arp2/3 and Cdc42/WASP/Arp2/3. BepC/F-dependent invasome formation further required cofilin 1, a protein that controls actin turnover. Based on these results, Truttmann et al. proposed that BepC and BepF would manipulate host signaling at an intermediate stage of the cascade leading to invasome formation, while BepG would operate more downstream and potentially interact with actin itself, thus making cofilin 1 dispensable (358, 420). Although no direct evidence corroborates a role for the particular process of invasome formation in *Bartonella* infections *in vivo*, the similarity to the bacterial aggregates in the surrounding of proliferating endothelial cells in bacillary angiomatosis lesions is striking (117).

In addition to invasome formation, it was further noticed that *B. henselae* Houston-1 shows clear VirB/D4-dependent stimulation of angiogenesis in addition to a basal VirB/D4-independent proangiogenic activity and thus apparently contributes to the contact-dependent activities described above. Using an *in vitro* 3D model for angiogenesis that followed the cumulative length of capillaries sprouting out of endothelial cell spheroids, it was found that the VirB/D4-dependent stimulation was in fact the net effect of Beps with pro- and antiangiogenic properties: while BepA (heavily) and BepD (moderately) promoted sprouting when expressed in an effectorless mutant upon infection, BepG strongly inhibited angiogenesis in this model and even diminished the VirB/D4-independent stimulatory activity to the background of uninfected cells. Interestingly, the BID domain of BepA alone was sufficient to trigger the capillary sprouting phenotype (381). The authors also showed that *B. henselae* Houston-1 stimulated angiogenesis in their *in vitro* model to a similar extent as VEGF. Surprisingly, further investigation revealed that only the VirB/VirD4-independent angiogenic activity alone was cumulative with VEGF stimulation but that translocation of *Bartonella* effector proteins apparently made endothelial cells irresponsive to this growth factor (382). It could be shown that a protein tyrosine phosphatase (PTP) activity induced and/or provided by the Beps dephosphorylates at least two tyrosines in the cytosolic C terminus of VEGF-R2 (Tyr951 and Tyr1175). Phosphorylation of these tyrosines provides docking sites for downstream effectors of VEGF signaling, and both were shown to be either directly or indirectly involved in angiogenesis (187, 375, 449). In line with these findings, the authors demonstrated a corresponding obstruction of VEGF-R2 downstream signaling and further detected a considerable loss of cellular tyrosine phosphorylation at various molecular weights, indicating that VEGF-R2 would not be the only target of the effector-associated PTP activity. No single Bep was sufficient to restore the inhibition of VEGF signaling in an effectorless mutant, further highlighting that the effector proteins of *Bartonella* act together as a multifaceted cocktail that subverts host cell signaling.

In addition to its strong effect on angiogenesis, the BID domain of BepA was further shown to be responsible for an antiapoptotic activity observed during infections of human endothelial cells with *B. henselae* Houston-1 (384). Since endothelial cell proliferation is intimately connected to the inhibition of apoptosis (see above), it is likely that the two *in vitro* phenotypes of BepA arise from the same molecular activity. The particular mechanism underlying the effects of BepA has not been resolved, but inhibition of apoptosis correlates with its localization to the host cell plasma membrane and an increase in cellular cAMP levels (384). Experiments with drugs triggering elevated cAMP levels showed that such elevation could mimic the antiapoptotic effect of BepA, suggesting that the increase in cellular cAMP observed in the presence of BepA is no side effect but the actual signal that mediates its antiapoptotic activity. Interestingly, the authors further reported that the BID domain of *B. quintana* BepA, but not the ortholog of *B. tribocorum*, inhibited apoptosis in human endothelial cells. These results are in line with experiments of Kirby and Nekorchuk that revealed a certain host-pathogen specificity of *Bartonella*-derived antiapoptotic and mitogenic effects (226). Furthermore, a recent study reported that the stimulation of cAMP production in endothelial cells was observed only when the Houston-1 strain of *B. henselae* had been used to infect (immortalized) HUVECs and not with any other combination of bacterial strain and host cell line (37), thus confirming that the cellular effects of the Beps are apparently in a subtle balance with other bacterial and/or host factors.

The proinflammatory activation of endothelial cells via activities of *Bartonella* effector proteins comes in addition to a VirB/D4-independent activation of NF-κB, which has been linked to several virulence factors such as outer membrane proteins (OMPs) or BadA of *B. henselae* (385). The VirB/D4-dependent increase in NF-κB activation was evident in a corresponding elevation of ICAM-1 surface expression and IL-8 secretion, supporting the idea that the proinflammatory effect may overlap with or be part of the angiogenic activity of the *Bartonella* effector proteins and participate in the dedicated contact-dependent effects on endothelial cells.

Since *Bartonella* research in the last decades focused on *B. bacilliformis*, *B. henselae*, and *B. quintana* due to their role as human pathogens, suitable *in vitro* infection models for species of lineage 3 are not available. No evidence for the translocation of these Beps into host cells or for potential cellular activities or phenotypes (apart from the phosphorylation of tyrosine motifs) is available. However, future research may elucidate the role of the VirB/Bep system in infections of these species and allow us to explore the parallel evolution of *Bartonella* effector proteins at a cellular level.

(d) Possible functional interaction with TAAs. It has been speculated for *B. henselae* that BadA and the VirB/D4 T4SS might interact synergistically in a way that BadA would mediate initial attachment to target cells, facilitate effector secretion, and manipulate cellular functions together with the Beps (322). The concept of such a relationship between an adhesin and a T4SS is reminiscent of the case for *Helicobacter pylori*, where host cell binding via the BabA protein was shown to amplify phenotypes associated with effector secretion (199). Although one could imagine that the enormous size and the dense surface layer of BadA may interfere with *Bartonella* effector secretion, the T-pilus of the plant-targeting *A. tumefaciens* VirB/D4 T4SS is known to range from 1.4 μm length on average up to even 4.5 μm and would hence be much longer than BadA (8). It is true that the participation of a T4S pilus in *Bartonella* effector secretion has never been demonstrated, but conjugation of the prototypic *Escherichia coli* F plasmid occurs over a distance of up to 12 μm (18), confirming that T4S is able to bridge remarkable distances and may be able to cross the BadA surface layer. As an alternative to a functional interaction, it was hypothesized that BadA and the VirB/D4 T4SS may not be coexpressed in the bacteria (216). At least a certain regulatory antagonism is indeed evident from transcriptional data on *B. henselae* during infections *in vitro*, since under these conditions expression of the VirB/D4 T4SS was upregulated and that of BadA downregulated (347). In conclusion, it seems plausible that BadA and the VirB/D4 T4SS of *B. henselae* could somehow cooperate in the manipulation of host cells, but experimental evidence supporting this hypothesis is missing.

The direct investigation of the potential functional interaction between BadA and the VirB/D4 T4SS has so far been impeded by the use of strains that express only one of the two (see above). Since assembly of both macromolecular machineries is very costly for the bacterium, one factor is typically lost under nonselecting conditions in the laboratory, although published reports are available only for loss of BadA expression *in vitro* (225, 361).

(ii) The Vbh T4SS. The genome sequence of *B. tribocorum* revealed the presence of a third type IV secretion system in addition to those encoded by the *virB/D4* and *trw* loci that was, owing to considerable protein sequence identity of 40 to 80%, called the VirB-homologous (Vbh) T4SS. Subsequent phylogenetic analyses showed that the Vbh T4SS and the VirB/D4 T4SS cluster together in a clade that is clearly separate but most related to a set of conjugative machineries in other *Rhizobiales*, such as the AvhB T4SS in the pATC58 plasmid of *A. tumefaciens* (374). Close homologs of the Vbh T4SS were found in other *Bartonella* species (34, 134), and although no direct evidence corroborates a function in virulence, its presence in all species of lineage 2 as the sole T4SS has been interpreted as indicative of a role in pathogenesis (115, 374). This hypothesis was further supported by the discovery of genes coding for potential effectors with a FIC-BID domain architecture close to the *vbh* loci in *B. grahamii* and *B. schoenbuchensis* (34, 134). Elucidating a potential function of the Vbh T4SS in the pathogenesis of *Bartonella* will provide important insight into both the virulence and evolution of this genus.

Outer membrane proteins. A study with *B. henselae* revealed that various outer membrane proteins (of 28 kDa, 32 kDa, 43 kDa, 52 kDa, and 58 kDa in size) bind human endothelial cells *in vitro*, indicating that they could act as adhesins or somehow stably interact with cell surface moieties (64). While the 43-kDa protein was found to be the strongest adhesin and may very well be OMP43 (see "Other Virulence Factors" below), another study discovered that low-molecular-mass outer membrane proteins (3 to 33 kDa) of *B. henselae* Houston-1 were sufficient to trigger NF-κB activation independent of LPS and Toll-like receptor 4 (TLR4) and stimulated the secretion of MCP-1 by endothelial cells (282). Consistent with this, another group reported that outer membrane proteins of *B. henselae* Berlin-1 and *B. henselae* Houston-1 could activate NF-κB in HUVECs, leading to the upregulation of adhesion molecules (E-selectin and ICAM-1) that may promote the attachment of circulating leukocytes (148). The interaction partners of these *Bartonella* outer membrane proteins on the host cell surface are not known, but it has been suggested that ICAM-1 may be one of them, since this protein is enriched in the tips of host cell membrane protrusions that tightly associate with the bacterial aggregates during invasome formation (120). It is further tempting to speculate that one or more of the host cell binding outer membrane proteins would be the elusive factor of *B. henselae* Houston-1 that binds β1-integrins in the context of invasome formation and is regulated by the BatR/BatS system (419).

Taken together, current knowledge suggests that one or more outer membrane proteins of *Bartonella* contribute to host cell adhesion and the contact-dependent proinflammatory activation of endothelial cells that was observed *in vitro* and *in vivo*, although the identity of these proteins has remained elusive so far. More outer membrane proteins of *Bartonella* that are suspected to be virulence factors but whose particular roles during infection have not been worked out are discussed in "Other Virulence Factors" below.

GroEL. Bacterial GroEL heat shock proteins are a conserved group of chaperonins whose function to mediate protein folding has made them widely essential to resist various stressors such as extreme temperatures or nutrient limitation (164, 265, 348). Interestingly, although no member of the GroEL family bears any discernible secretion signal, overwhelming evidence indicates that surface-exposed or secreted GroEL plays a key role in the infection strategies of many bacterial pathogens (35, 181, 184; reviewed in reference 174). As an example, the corresponding homolog of *Legionella pneumophila* is known to be displayed on the bacterial surface, where it promotes an invasive phenotype, and the same protein apparently manipulates cellular trafficking, cytoskeleton, and signaling once it is released inside the eukaryotic host cell (recently reviewed in reference 153).

It is therefore not surprising that GroEL of *Bartonella* is strongly suspected to be a virulence factor, although its role during infection is not well understood. Most evidence derives from experiments with *B. bacilliformis*, where GroEL was found not only in the cytoplasm but also in the outer membrane and actively secreted into culture supernatants (297). These supernatants as well as lysates of *B. bacilliformis* triggered the proliferation of HUVECs, indicating the presence of a mitogen, and antibodies against GroEL or its partner GroES strongly reduced this activity. The presence of a dose-dependent mitogen in extracts of this species had already been discovered before (150, 151), and lysates of *B. bacilliformis* expressing different amounts of GroEL triggered cell proliferation in accord with GroEL levels (297). This stimulatory effect of GroEL on host cell proliferation is apparently a conserved element in the infection strategy of *Bartonella*, since lysates generated from *B. henselae* also showed mitogenic activity, albeit weaker than that of lysates of *B. bacilliformis*. These results are in line with other reports that *B. henselae* secretes an antiapoptotic and mitogenic activity into culture supernatants (226, 268) that may very well be GroEL. Interestingly, the antiapoptotic activity of these supernatants was weaker than the net effect of live *B. henselae*, hence confirming the role of additional factors that are known to act on the same phenotype and may account for an attenuation of GroEL potency during evolution (like the VirB/D4 T4SS). GroEL was further found to localize to the outer membrane not only of *B. bacilliformis* but also of *B. henselae*, and it is highly immunogenic in various *Bartonella* species (43, 84, 229, 357, 432). In addition, it was recognized that *Bartonella* GroEL harbors a C-terminal phenylalanine that is a general characteristic of outer membrane proteins, indicating that GroEL of *Bartonella* would be adapted to exposure on the bacterial surface (69, 409).

It remains enigmatic how GroEL of *Bartonella* would act as a mitogenic and antiapoptotic factor on the molecular level, but corresponding activity has been shown for GroEL of *Chlamydia pneumoniae*, which triggers the proliferation of human cells via TLR4-dependent activation of p44/p42 mitogen-activated protein kinases (MAPKs) (380). GroEL could hence be imagined to directly trigger proliferative signaling and/or to induce the secretion of factors that act as autocrine or paracrine mitogens such as IL-8. The latter hypothesis is consistent with findings that *E. coli* GroEL stimulates the expression of granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, and ICAM-1 in HUVECs (149). A recent study indeed reported that the secreted mitogen of *B. henselae*, most likely GroEL, would act via an elevation of the intracellular Ca^{2+} levels (283). Alternatively, the effect of *Bartonella* GroEL could be indirect, for example, by protecting the actual yet unknown bacterial mitogen from degradation.

Due to its essential role in cellular homeostasis, the importance of GroEL for *Bartonella* pathogenesis could not be assessed in the STM screens with *B. birtlesii* or *B. tribocorum*, but GroEL was found to be part of the BatR/BatS virulence regulon in *B. henselae* (347). Surprisingly, the *groEL* gene seems to be strongly downregulated during *in vitro* infection of endothelial cells, which is bizarre with respect to its major role both in stress responses and as a potential virulence factor, but could protect heavily infected cells from adverse effects. It was further reported that, while mitogenicity had initially been observed with lysates of *B. bacilliformis*, the coculturing of live bacteria stimulated HUVEC proliferation only with strains expressing low or moderate levels of GroEL. Expression of large amounts of GroEL appeared to induce apoptosis in HUVECs after long coculture (96 h), although all strains initially inhibited spontaneous apoptosis (407). The authors conclusively interpret the apparent contradiction to previous results with the homology of bacterial GroEL to eukaryotic HSP60 chaperonins that are known to stimulate apoptosis if present in huge excess. The proapoptotic effect in coculture would hence derive from intracellular bacteria releasing large amounts of GroEL, while the stimulatory effect on cell proliferation would most likely be triggered by extracellular GroEL (407). It is thus tempting to speculate that the potentially deleterious effects of intracellular GroEL secretion in heavily infected cells could be counteracted under physiological conditions by the downregulation of bacterial *groEL* expression that was described for *B. henselae*.

Taken together, solid evidence suggests that GroEL is the secreted mitogenic and antiapoptotic factor of *Bartonella* that has been described repeatedly, although the underlying molecular mechanism remains unknown.

Infection of Erythrocytes

The Trw T4SS. In addition to the VirB/D4 T4SS, the species of lineage 4 harbor the Trw T4SS as a lineage-specific virulence factor that mediates adhesion to red blood cells (427). It is not closely related to the other type IV secretion systems of *Bartonella* but has apparently been acquired independently. Sequence similarity of up to 80% at the protein level to the conjugative Trw T4SS of the IncW broad-host-range plasmid R388 suggests that it may have been obtained from such a conjugative plasmid via lateral gene transfer, especially since transcriptional regulation by the KorA/KorB repressor system is conserved in *Bartonella* (402).

The Trw T4SS was discovered using a differential fluorescence induction (DFI) screen for genes that are upregulated in *B. henselae* during infection of endothelial cells *in vitro*, and the STM screens in *B. birtlesii* and *B. tribocorum* confirmed its essentiality as a virulence factor for the species of lineage 4 (374, 427). Interestingly, a mutant devoid of a functional Trw system was found to appear in the bloodstream but was cleared shortly after infection, which provided the first evidence that the Trw T4SS is involved in erythrocyte infection rather than colonization of the primary niche (116). The upregulation observed during endothelial cell infection hence apparently constitutes a phenotypic adaptation of the bacteria for the infection of red blood cells, since expression and assembly of the Trw machinery only in the bloodstream would extend the time that the bacteria had to spend in this hostile environment. Further investigation revealed that the Trw T4SS not only is essential for the adhesion of *Bartonella* to erythrocytes but also dictates the host specificity of this process, most likely by adaptation to erythrocyte surface structures that are highly variable among the different host species of the bartonellae. Strikingly, ectopic expression of the Trw T4SS of one *Bartonella* species in another one was found to be sufficient for a corresponding extension of the host range in an *in vitro* erythrocyte infection assay (427).

In contrast to the VirB/D4 T4SS of *Bartonella*, the Trw T4SS apparently neither harbors a coupling protein nor translocates any known effectors, but it carries numerous tandem gene duplications coding mostly for surface-exposed subunits (401). Coduplicated gene copies encoding a core subunit are highly conserved, while the genes encoding putative minor and major pilus subunits have been shaped by diversifying selection. Diversification is thought to have occurred via intra- and intergenomic combinatorial sequence reshuffling and even led to potential subfunctionalization into two versions of the major pilus subunit. It is assumed that the polymorphicity of erythrocyte surface structures in mammals has been the driving force for this remarkable sequence dispersion (316), especially since the different copies of Trw surface proteins seem to be coexpressed (115). Such host polymorphisms have also been proposed to affect red blood cell invasion of *Plasmodium* parasites (289, 414, 437), but it remains unknown which host factors the Trw T4SS binds on the erythrocyte surface.

Deformin. Evaluation of human red blood cells infected with *B. bacilliformis in vitro* revealed deformations appearing as "pits" or "trenches" on the erythrocyte surface (31), which were proposed to serve as entry points for the invasion process (Fig. 5). It has been suggested that a secreted bacterial factor, deformin, would cause the membrane invaginations (440), but its molecular identity, mode of action, and secretion mechanism have remained elusive so far, although a well-documented phenomenology allows us to assemble a basic picture of its functionality. Importantly, deformation could also be demonstrated for *B. henselae*, making it likely to be a common feature of *Bartonella* erythrocyte infection (201).

Early reports describing deformin to be a protein of 65 kDa or 130 kDa (440) or implying physicochemical properties of proteins (e.g., heat inactivation [287]) arose from confusion with its tight binding to serum albumin. Instead, deformin was found to be a small (1.4-kDa) water-soluble but amphiphilic molecule that is resistant to heat, several proteases, α-amylases, and pH change in the range of 2 to 9.5 (126).

Filtered supernatants of *B. bacilliformis* cultured in certain media could reproduce the deformation phenotype in the absence of bacteria, since they apparently contained deformin bound to serum albumin (287). The pretreatment of erythrocytes with trypsin or neuraminidase greatly favored deformation, and without such pretreatment the deformation phenotype induced by deformin in filtered culture supernatant was very weak compared to that of whole, live bacteria (287). These results suggest that increased accessibility of erythrocyte membranes could facilitate the activity of deformin and that live bacteria may harbor additional factors, like a secretion machinery and/or hydrolytic enzymes, that would assist deformin delivery. It is worth noting that pretreatment with trypsin or neuraminidase also strongly enhanced the binding of *B. bacilliformis* to glycophorin B from solubilized erythrocytes (63), although the link to deformation is not clear. Additional evidence for a manipulation of erythrocyte membranes as underlying red

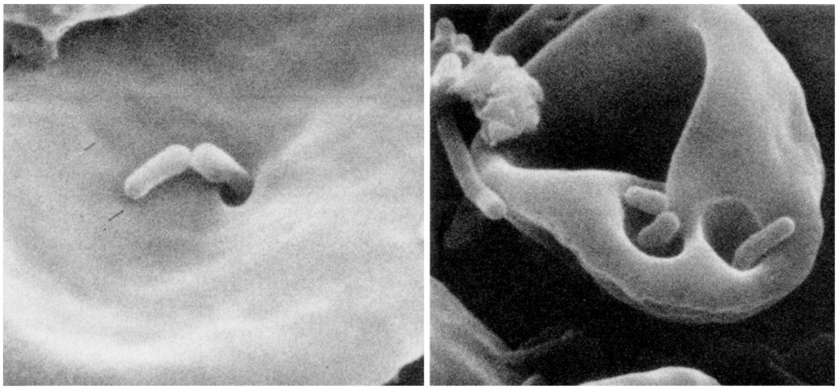


FIG 5 Entry of *Bartonella* into human erythrocytes. The scanning electron micrographs show *B. bacilliformis* first inducing indentations in the erythrocyte surface (left) and then invading at the resulting pits (right). Note that neither the deformation of erythrocyte membranes nor the actual invasion process appears to involve rupturing of the erythrocyte surface. (Adapted from reference 31 with permission.)

blood cell deformation comes from findings that phospholipase D (cleaving phosphatidylcholine) and vanadate (which inhibits the flippase that maintains membrane leaflet asymmetry [332]) dampen and prevent deformation, respectively (287, 440).

The function of erythrocyte deformation during blood-stage infection by *B. bacilliformis* has not been resolved experimentally, but the hypothesis of it being involved in the formation of some kind of entry site is convincing (295), especially since deformation was found to precede erythrocyte invasion (287).

No molecular mechanism has been demonstrated so far for *Bartonella*-induced red blood cell deformation, but the small size of deformin and the finding that it does not directly modify phospholipids (440) speak against a biochemical activity on membrane components. Interestingly, deformation is stable for some time but can be rapidly reversed by different compounds that interfere with the asymmetric distribution of phospholipids in the two membrane leaflets, indicating that deformin would exploit this asymmetry (440). Convincingly, these results let Xu et al. (440) speculate that deformin-induced membrane invaginations might arise in a way similar to what Sheetz and Singer proposed for erythrocyte stomatocytosis in their bilayer couple hypothesis (404). In short, invaginations on erythrocyte membranes could be induced by processes that would locally jumble the inner and outer leaflets (like intercalation of an amphipath), since active reconstitution of leaflet asymmetry occurs only from outside to inside (through the flippase mentioned above). This imbalance in lipid transport results in net extension of the inner leaflet, formation of an indentation, and finally vacuolization (259, 390). Although the molecular identity of deformin remains mysterious, a small peptide or lipid/LPS compound could be imagined to trigger deformation via processes like those described above. Importantly, deformation seems not to involve detectable rupturing of membrane integrity, because traversing diffusion of dyes or labeled proteins was never observed. Instead, the presence of deformin in the absence of bacteria was sufficient to trigger the uptake of extracellular fluid into intraerythrocytic vesicles, reminiscent of *Bartonella* “forced endocytosis” (31, 440). In further consistency with a mechanism involving integration of an amphipath into erythrocyte membranes, deformin seems to be consumed upon contact with red blood cells, since filtered supernatants with deformin were found to lose activity during serial addition and removal of erythrocytes (287). The whole process is strongly reminiscent of studies discussing the use of amphiphilic substances to trigger the uptake of drugs into intraerythrocytic vesicles (example in reference 389). Interestingly, such techniques were developed to deliver drugs to cells of the mononuclear phagocyte system, because these would selectively eliminate the abnormally vacuolized erythrocytes just as observed during Oroya fever.

Like most aspects of its activity, the mechanism by which deformin gets into the extracellular space has remained enigmatic. Remarkably, the apparent secretion of deformin into culture supernatants seems to depend on the presence of functional albumin or other serum components, because heating or dialysis of the culture medium after the addition of serum supplement made it incapable of acquiring deforming activity (287). It hence cannot be ruled out that the secretion observed *in vitro* may be nonphysiological. In any case, the presence of deformin in filtered supernatants was accompanied by bacterial outer membrane proteins appearing in association with membrane fragments or vesicles (440). Interestingly, the presence of bacterial membrane proteins of 31 to 36 kDa in supernatants generated with the KC583 strain of *B. bacilliformis* correlated with its ability to secrete deformin, while these proteins as well as deforming activity were missing in supernatants of the KC584 strain. Live bacteria of both strains induced deformation of erythrocytes (179), indicating that the discrepancy was due to differences in the secretion of deformin. The authors of the study proposed that the proteins missing in the supernatant of KC584 might be identical to deformin, involved in its secretion, or part of a “deforming protein complex” (179), but no direct link to deformation could be demonstrated experimentally. It is conceivable that these elusive proteins may derive from outer membrane vesicles (OMVs), which are a general phenomenon among Gram-negative bacteria (243) and often serve as vectors for the delivery of periplasmic or membrane-associated virulence factors to target cells (9, 290, 433). Alternatively, the proteins and membrane fragments may derive from bacteriophage-like particles, because their size and the association with some kind of membrane vesicles are immediately reminiscent of the BLPs that have been repeatedly described for *Bartonella* (see above). However, it remains enigmatic how the release of BLPs could be connected to the secretion of deformin, especially since both the KC583 and KC584 strains have been shown to harbor these particles (11).

In conclusion, it is apparent that membrane deformation is

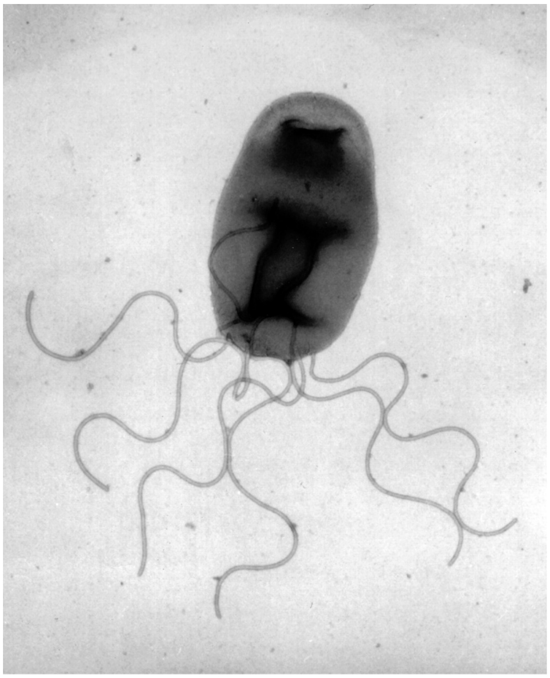


FIG 6 Flagellation of *Bartonella*. The transmission electron micrograph shows several unipolar (lophotrichous) flagella of *B. clarridgeiae*. Morphologically indistinguishable flagella have also been observed for other species of lineages 2 and 3 as well as *B. bacilliformis*, while the species of lineage 4 are not flagellated (31, 36, 118, 136). (Adapted from reference 13 with permission.)

important at an initial step of erythrocyte invasion during *Bartonella* infections, but the molecular mechanism and the identity of the deforming factor remain mysterious.

Flagella. In a remarkable difference from the species of lineage 4, the bartonellae of lineages 1 to 3 harbor a set of unipolar (lophotrichous) flagella and are thus motile (Fig. 6). Flagella are well established as bacterial virulence factors (210), and those of the flagellated bartonellae are known to play an important role during the infection of erythrocytes by these species. Early studies noted that nonmotile *B. bacilliformis* organisms were unable to invade red blood cells (287), and Benson et al. observed that bacteria were pushing into surface indentations with a "boring or twisting motion" (31), implying that the flagella might contribute mechanical force to the entry process. Nonmotile *B. bacilliformis* showed decreased erythrocyte attachment and deformation, indicating that motility would be beneficial but not necessary for these initial steps of red blood cell infection (287). Consistently, it was reported that antiflagellin antibodies decreased erythrocyte attachment but much more invasion of *B. bacilliformis* (383). It was further hypothesized that the flagella of *Bartonella* could play a role as adhesins like in the case of other pathogens (135), especially since fiber-like structures were apparent on bacteria attached to red blood cells (434). However, the differential effect of antiflagellin antibodies on adhesion and invasion makes such functionality unlikely (383), and it was reported that neither recombinant flagellin nor flagella isolated from *B. bacilliformis* could bind erythrocytes (193). Remarkably, a study using immunofluorescence to follow the flagella of *B. bacilliformis* during erythrocyte infection indicated that the flagella would be ejected for intraerythrocytic persistence (370), although the reasoning for this step remains unclear.

The apparent exchange of flagellation for the Trw T4SS in the species of lineage 4 and the common function of both virulence factors in erythrocyte invasion indicate that the Trw T4SS may have somehow functionally replaced the flagella directly or indirectly (115). Such a process could have been favored in evolution because the versatility of the Trw T4SS was of great advantage and/or because of disadvantages inherent to flagellation. It is particularly tempting to speculate that flagella would have been lost due to their role as major antigens of both innate and adaptive immune responses (253). Consistently, flagella of *Bartonella* may evade detection by the dedicated Toll-like receptor 5 (10), but they were found to elicit an antibody response (378) that would presumably heavily interfere with erythrocyte invasion (383) and clear the bacteria from the bloodstream.

Invasion-associated locus. The invasion-associated locus (*ial*), encoding IalA and IalB, is an essential virulence factor of *Bartonella* and critical for erythrocyte invasion but not adhesion (374, 427). Both proteins are conserved over the whole genus and were found to be sufficient for conferring a correspondingly invasive phenotype to ectopically expressing *Escherichia coli* (300).

IalA has been identified as a Nudix family nucleoside polyphosphate hydrolase (76, 101). These enzymes are involved in the catabolism of alarmones, and homologs of IalA in other pathogens were shown to be crucial for infectivity (38, 128, 200, 422). It is thus reasonable to assume that IalA may contribute indirectly to invasion, and a homolog in *Rickettsia prowazekii* was indeed suggested to "buffer" the bacterial homeostasis against stressful conditions during growth in a hostile, intracellular environment (154).

Conspicuously, the protein encoded directly upstream of IalA, CtpA, is a C-terminal processing protease that has initially been proposed to control levels of protein junk under stress conditions during infection (299). Homologs of CtpA are important virulence factors of other pathogens (27, 353), and the CtpA protein of *Borrelia burgdorferi* was found to process several outer membrane proteins known or thought to be involved in pathogenesis (323, 338, 451). However, the initial publication concerned with CtpA in *Bartonella* failed to detect processing of IalA or IalB by CtpA or any effect of CtpA on erythrocyte invasion (299), meaning that functional evidence for a contribution of this protein to *Bartonella* virulence is missing. A role for pathogenicity in *Bartonella* seems still likely, since, e.g., the ortholog in the closely related *Brucella suis* was shown to be necessary for survival in macrophages *in vitro* and infection of mice *in vivo* (22). Similarly, the *filA* gene just upstream of *ctpA* has also been suspected to encode a virulence factor, but no experimental findings that would confirm or discard this assumption have been published (293). It was, however, shown that the promoter of *filA* contains an H box that is indicative of transcriptional regulation by Irr together with other virulence factors such as the hemin binding proteins or OMP43 (26).

In contrast to IalA and CtpA, IalB is an outer membrane protein based on its signal sequence and findings in *B. henselae* (84), thus seeming to be the natural candidate for the host-interacting component of the invasion-associated locus. Initial results that IalB of *B. bacilliformis* was located in the inner membrane were surprising for the authors of the study as well and could have been caused by suboptimal growth conditions (100). The crystal structure of IalB of *B. henselae* was solved recently by Patskovsky et al. and revealed a β-barrel fold (Protein Data Bank [PDB] no. 3DTD), further confirming localization in the outer membrane

(232). Although homologs of IalB are widely conserved, especially among *Rhizobiales*, including the zoonotic pathogen *Brucella suis* (333), possible enzymatic capacities and/or host interaction partners remain elusive. However, IalB was described to exhibit considerable sequence similarity to the *Yersinia* Ail protein, which belongs to a family of bacterial outer membrane proteins whose members have frequently been associated with host cell attachment and invasion (172, 300). It is thus easily conceivable that IalB may be directly involved in erythrocyte invasion, possibly by interacting with one or more of the red blood cell surface proteins bound by *Bartonella*.

Transcriptional analysis of *B. bacilliformis* showed that IalB expression is strongly upregulated under conditions resembling the sand fly gut (20°C and acidic pH), suggesting that the bacteria would become "primed" for erythrocyte infection in the arthropod vector (99). Mutants lacking functional IalB both in *B. bacilliformis* (100) and in *B. birtlesii* (427) have been tested using *in vitro* assays for erythrocyte infection and were strongly attenuated compared to wild-type bacteria. Taken together, these results indicate that IalB and the invasion-associated locus are vertically inherited key virulence factors of *Bartonella* whose function is essential for erythrocyte invasion.

Hemolysins. The acute phase of infections with *B. bacilliformis* is characterized by severe hemolytic anemia that accounts for high mortality rates in untreated patients and is usually not associated with infections caused by the modern *Bartonella* species.

In apparent contradiction to several studies concluding that *B. bacilliformis* does not actively lyse erythrocytes during infection (see above), one study reported the discovery of a membrane-associated, proteic hemolysin in *B. bacilliformis* that was absent in *B. henselae* (178). The author states that hemolysis was contact dependent and not connected to the process of erythrocyte deformation. It apparently did not rupture erythrocytes but rather led to the formation of intact ghosts, indicating that hemolysis may derive from some kind of local puncture. However, other studies explicitly did not detect a loss of membrane integrity in infected erythrocytes (31). It is possible that this inconsistency derives from differences in the experimental conditions, since the author reporting hemolysis pointed out that such a phenomenon was not presentably detectable on blood agar plates but required an elaborate experimental procedure (178). We therefore suggest that hemolysis during Oroya fever may not be caused primarily by a bacterial hemolysin, although a membranolytic factor could allow *Bartonella* (*B. bacilliformis*) to escape from intracellular vesicles at any state of infection and still, under certain conditions, lyse red blood cells *in vitro*.

Although hemolysis has been reported as a typical feature only of *B. bacilliformis*, all species of the genus *Bartonella* seem to carry genes that are annotated as encoding putative hemolysins, such as, e.g., the *tlyA* or *tlyC* homologs in *B. henselae*. However, no evidence for a role of these elusive factors in the infection process of *Bartonella* has been published. More solid data are available for one autotransporter of *B. henselae*, termed Cfa (CAMP-like factor autotransporter), which was found to bear cohemolysin activity, i.e., the capacity to disrupt membranes of predestabilized erythrocytes (261). The fact that orthologs of this protein were shown to be essential for *Bartonella* pathogenesis in both STM screens confirms its importance as a virulence factor (374, 427). However, the Cfa mutant of the *B. birtlesii* screen was apparently not impaired in erythrocyte infection *in vitro*, indicating that it may act at a different stage of infection (427).

Other Virulence Factors

Lipopolysaccharides. It is a critical requirement for the stealth infection strategy of any Gram-negative bacterial pathogen that the host cannot detect its lipopolysaccharides (LPS), since these are among the universal properties of bacterial pathogens that are recognized by innate immunity. Stimulation of Toll-like receptor 4 (TLR4) with LPS triggers a strong inflammatory response (291) that would likely obstruct bacterial persistence. It is therefore not surprising that the LPS of *Bartonella* are adapted to virtual invisibility to the host's defenses and have even been shown to participate in the immunomodulation that is one of the most amazing features of this group of pathogens. In addition to these aspects directly linked to immunity, the low endotoxicity of *Bartonella* LPS was suggested to be critical for the intimate interaction with endotoxin-sensitive cells such as endothelial cells (448).

Most research on the LPS of *Bartonella* has been performed with *B. quintana*, and the LPS of this species were shown to stimulate TLR4 three to four orders of magnitude less than *Salmonella* LPS, so TLR4 apparently does not play a role in the recognition of this pathogen (277). TLR2, a peculiar receptor recognizing primarily lipoproteins and lipopeptides (447), was found to be unresponsive to *B. quintana* LPS but apparently reacts to LPS-associated bacterial components (see below). Furthermore, it was shown that the LPS of *B. bacilliformis*, *B. henselae*, and *B. quintana* do not elicit a specific humoral immune response in mammals (84, 292).

In apparent contradiction to their definitive role in immune evasion, the LPS of *B. quintana* reacted strongly in the *Limulus* amebocyte lysate assay, a standard tool to detect endotoxicity (278). However, no considerable secretion of TNF-α could be detected in human whole blood challenged with *B. quintana* LPS or upon intravenous injection of the LPS into rats (278). This striking lack of proinflammatory activation is likely based on the remarkable property of *B. quintana* LPS of not only being virtually undetectable by TLR4 but even acting as a potent antagonist of TLR4 signaling, leading to considerable transcriptional downregulation of the proinflammatory program triggered upon activation of this receptor (including TNF-α) (340). In addition to the silencing of TLR4, an apparently LPS-associated component of *B. quintana* stimulated the secretion of IL-8, IL-10, and (small amounts of) TNF-α in host cells (277, 278). Consistently, TLR2-dependent production of IL-8 was also observed for *B. henselae* crude LPS extracts but was not detected upon further purification (277), which could explain the stimulation of IL-8 secretion in rats injected with *B. quintana* LPS as reported in an earlier study (278). Taken together, the available evidence shows that the LPS of *Bartonella* have immunomodulatory properties by acting as TLR4 antagonists and that an LPS-associated factor (perhaps an outer membrane lipoprotein) can obviously trigger cytokine secretion via TLR2 as part of the contact-dependent proangiogenic activities of *Bartonella*.

These immunoevasive and immunomodulatory properties of *Bartonella* LPS apparently derive from their molecular architecture. The structure of the *B. henselae* LPS has been solved by Zähringer et al. and revealed a deep-rough chemotype, in line with findings for other *Bartonella* species such as *B. bacilliformis* or *B. quintana* (448). In addition, the LPS of *B. henselae* is pentaacylated and contains a very small carbohydrate moiety and a long-chain fatty acid, which are well-known attributes of LPS of other stealth pathogens such as *Legionella* (446), indicating that these features may enable immune evasion by common mechanisms (448). It is worth noting that the *Bartonella* LPS do not seem to be homogenously deep-rough, since Zähringer et al. reported a certain proportion of smooth LPS and linked their finding to the smooth and dry colony phenotypes observed during growth of *Bartonella* on plates (448). The dimorphism between "dry agar-pitting" and "smooth non-agar-pitting" colonies has been observed for several *Bartonella* species, such as *B. henselae* (24), *B. schoenbuchensis* (118), and *B. bacilliformis* (434) and is believed to be caused by phase variation, although the molecular basis of this phenomenon has never been revealed (245). No function for the smooth LPS of *Bartonella* has been worked out so far, but a similar phenomenon of LPS heterogeneity is known from other *Rhizobiales* such as *Sinorhizobium*, where it was suggested to play a role in host interaction (163).

Autotransporters and filamentous hemagglutinins. In addition to trimeric autotransporter adhesins like BadA, the bartonellae also harbor classical autotransporters as well as two-partner secretion systems and hence feature representatives of each class of type V secretion systems (177).

Classical autotransporters are similar to TAAs in that an N-terminal passenger domain is translocated over the outer membrane through a C-terminal transporter domain, but they contain a full β-barrel transporter per monomer. The passenger domain is usually cleaved off from the transporter after translocation but typically stays attached to the bacterial surface (recently reviewed by Dautin and Bernstein [109]). Autotransporters are important virulence factors of various bacterial pathogens (176), but comparably little is known about the homologs in *Bartonella.* It is apparent that all *Bartonella* species encode a set of several autotransporters that belong to two distinct phylogenetic clades (personal observation). Representatives of the larger clade were first described as bacterial factors upregulated during *in vitro* infection of endothelial cells, and hence the genes were called *iba* (for inducible *Bartonella* autotransporters) (403). This clade of autotransporters was analyzed by Berglund et al. *in silico* and is again separated into two phylogenetic groups that seem monophyletic in *Bartonella* but separated before branching off of the *B. bacilliformis* lineage (34). Two representatives of the *iba* group were investigated by Litwin et al. and termed Arp (for acidic repeat protein) (262) and Cfa (for CAMP-like factor autotransporter) (261). While Cfa was found to exert cohemolysin activity (see above), the passenger domain of Arp shows sequence similarity to pertactin-like adhesins, which was interpreted as an indication of a potential related function of Arp (262). Notably, Arp and Cfa are representatives of the two distinct clades of *iba* autotransporters and were both found to stay attached to the outer membrane after cleavage from the transporter domain (261, 262), indicating that this may be a common feature of these proteins in *Bartonella*. Several *iba* autotransporters were confirmed to be upregulated during *in vitro* infections with *B. henselae* in a second study and were found to be essential for virulence in the two STM screens (e.g., Cfa) and immunogenic in infections (347, 374, 427, 432). Furthermore, representatives of this group in *B. quintana* were shown to contain an H box in their promoters, which indicates regulation in response to the transcription factor Irr together with other virulence-related genes, like those encoding the hemin binding proteins (26).

A second, smaller group of autotransporters (in *B. henselae* BH05490, BH05500, and BH05510) also features representatives in every *Bartonella* species but shows sequence similarity to autotransporters of *Enterobacteriales* such as *Yersinia* rather than to the *iba* proteins (personal observation). Like some *iba* genes, autotransporter genes of this group were upregulated during *in vitro* infection of human endothelial cells and were found to be immunogenic (347, 432), but they do not seem to be individually essential for establishing blood-stage infection as examined in the STM screens (374, 427).

Molecular functions or any particular contribution of both groups of autotransporters during *Bartonella* pathogenesis remain (with the partial exception of Cfa) to be uncovered. Other autotransporters of bacterial pathogens have been found to exert various functionalities, e.g., as adhesins (252) or as extracellular proteases for antibodies (298) or for hemoglobin (324).

The filamentous hemagglutinins (Fha) are an additional type V secretion system of *Bartonella* that is present only in *B. grahamii*, *B. henselae*, and *B. tribocorum* among the sequenced species (34). Filamentous hemagglutinins are secreted in a manner very similar to that for classical autotransporters, but the passenger domain (the actual filamentous hemagglutinin FhaB) and the transporter domain (FhaC or Hec for hemolysin activator protein) are encoded in separate genes. After translocation over the outer membrane, the passenger domain is usually subjected to further processing and finally either is secreted into the extracellular space or stays attached to the transporter or the outer membrane (96, 205, 281). The filamentous hemagglutinins of *Bartonella* can be separated into two clades that were apparently acquired independently from different sources. It is notable that the *fha* loci are associated with integrases and phage genes and that the distribution of their occurrence is very diverse even within closely related populations, pointing toward frequent exchange via lateral gene transfer (32, 34). Interestingly, *B. quintana* does not encode any filamentous hemagglutinins, although the closely related *B. henselae* harbors various copies of both passengers and transporters (34). The available data do not allow conclusive assignment of any function to the Fha proteins in *Bartonella* (322), but the genes coding for the outer membrane transporters were found to be regulated by the BatR/BatS system in *B. henselae* (347). One filamentous hemagglutinin and two transporters were immunogenic in cats infected with *B. henselae* (432), thus proving that at least some of these proteins are expressed inside the host. No *fha* gene is essential for the pathogenesis of *B. tribocorum* (374), but the presence of various *fhaB* and *hec* genes indicates potential redundancy that would impede detection by STM (322). The filamentous hemagglutinins may further be capable of extracellular complementation, especially since the passenger domain is potentially released from the bacterial surface.

Filamentous hemagglutinins are important virulence factors of other pathogens such as *Bordetella*, where they are necessary for optimal colonization of the respiratory tract (312) and may be key factors for host specificity (194). The *Bordetella* Fhas have been shown to mediate adhesion to various host structures (204) and to modulate the NF-κB pathway of immune cells (2). Similarly, an analysis of the filamentous hemagglutinins in *Erwinia chrysanthemi* revealed that they are required for full virulence and pointed toward horizontal gene transfer as a common feature in the spread of these pathogenicity factors (364).

Hemin binding proteins. The hemin binding proteins (Hbps) of *Bartonella* are a group of porin-like outer membrane proteins that bind hemin on the bacterial surface, but lack any discernible homology to known bacterial heme receptors (25). Remarkably, Pap31, the first known member of this family in *Bartonella*, was discovered not as a virulence factor but as being a major protein component of the BLPs that *Bartonella* releases into culture media, although it is known today that its prevalence in the phage-like particles is rather coincidental (see above). Subsequent research established the alternative denomination of Pap31 orthologs as HbpA and identified the Hbps as a protein family (75, 296). Three to five representatives are present in the genome of every *Bartonella* species, and homologs are widespread among *Rhizobiales* but rare beyond this taxon (reference 294 and personal observation). The Hbps show considerable sequence similarity to members of the OMP25/31 family of *Brucella* (75), which are apparently crucial for several aspects of pathogenesis, although their molecular function is not known (74, 276). Furthermore, homologies to the well-known *Neisseria* Opa proteins, which are critical mediators of host cell interaction involving invasion and immunomodulation (372, 388), have been described (296). Comprehensive theories explaining the contribution of *Bartonella* Hbps to pathogenesis are lacking, although a plethora of experimental data are available and various (not mutually exclusive) hypotheses have been proposed. In short, the Hbps were suggested to be (part of) a heme uptake system, to form an antioxidant coat, to maintain a microaerobic intracellular environment, or to participate directly in host cell manipulation (26).

It is undisputed that the acquisition of heme is critical for *Bartonella*, since the bartonellae have an exceptionally high need for this compound (377). However, the hemin binding activity of the Hbps does not automatically imply a function in heme acquisition, especially since *Bartonella* harbors a distinct, dedicated hemin import system (HutABC/HmuV) (331) (see below). One study reported that HbpA of *B. henselae*, whose ortholog in *B. quintana* constitutes the strongest hemin binding activity of this species (75), could complement a *hemA* deficiency in *E. coli* and thus act as part of a hemin uptake machinery (453). However, these results have been decisively challenged by other authors (294), especially since the *hemA*-deficient *E. coli* strains are known to display a leaky phenotype (379). We hence conclude that a primary function of the *Bartonella* hemin binding proteins in heme acquisition seems unlikely, although an accessory role via the accumulation of substrate is conceivable.

In addition to this potential facilitation of heme acquisition, the hemin binding of the Hbps should lead to a coat of heme on the cell surface, whose properties and functions would depend largely on the properties of heme. Extracellular deposition of heme is also seen in *Yersinia pestis*, where efficient transmission by bloodsucking arthropods depends on outer membrane storage of this compound (183, 337). Most obviously, such a surface coat would provide a nutritive reservoir for *Bartonella*, especially as it has to compete with its arthropod vector for heme in the midgut (25). Furthermore, the intrinsic peroxidase activity of heme would make the coat a potent antioxidant barrier (25) and hence constitute a suitable protectant against reactive oxygen species (ROS) as key mediators of antimicrobial defense both in the arthropod gut (169, 305) and as part of mammal immune defenses (42). ROS release in the arthropod gut is highly regulated, for example, in order to protect symbionts (168), and was found to be decreased in bloodsucking arthropods upon perception of heme in the gut lumen (319). Such a mechanism would potentially allow *Bartonella* not only to tolerate but also to prevent certain challenge by ROS, though no experimental data are available. *Porphyromonas gingivalis* is an example of a pathogen that uses surface coating with heme and related compounds as a defense mechanism against ROS in humans (405, 406). Similarly, heme can also modulate the behavior of mammalian cells (15, 127, 138), making it conceivable that a coat of heme would also contribute to subvert responses of the vertebrate host.

Besides functions in host interaction, heme would also decrease oxygen levels around the bacteria. Battisti et al. speculated that bartonellae (exemplified by *B. quintana*) would lack aerobic respiration and could, similarly to other *Rhizobiales* such as *Agrobacterium* spp., be adapted to growth in microaerobic compartments. They further proposed that hemin binding via Hbps or their orthologs may be a common strategy for this purpose among different *Rhizobiales* (25, 26).

Finally, HbpA of *B. henselae* was shown to bind fibronectin, heparin, and HUVECs (107). Binding to these ECM compounds has been linked to the homologous OpaA protein of *Neisseria*, which also binds fibronectin and mediates invasion of epithelial cells via an integrin-dependent mechanism (107, 425). Additionally, a *B. henselae* HbpD mutant was deficient in escape from phagosome maturation (246), but the underlying direct or indirect mechanism has not been resolved.

In summary, the abundance of evidence makes it likely that the Hbps contribute prominently to *Bartonella* pathogenesis in several ways. It is thus not surprising that studies of *B. quintana* discovered sophisticated transcriptional control mechanisms guiding *hbp* expression and revealed a clear correlation between biologically relevant stimuli and altered expression patterns of the *hbp* genes (25). From a regulatory point of view, the Hbps seem to constitute two subgroups, with the first one (HbpB and HbpC) being preferentially expressed under conditions that resemble the arthropod midgut or feces (high hemin and 30°C) and the second one (HbpA, HbpD, and HbpE) being preferentially expressed under mammal-like conditions (37°C and low hemin) (25). Although this second subgroup was still predominant under bloodstream-like conditions (5% instead of 21% O_2), the decrease in oxygen led to significant downregulation of all Hbps, indicating that these proteins might not be of major importance in the intraerythrocytic niche. Alterations of pH and free iron concentrations did not have significant effects on *hbp* expression (25).

Several transcription factors were found to exhibit differential expression in response to the environmental cues known to influence *hbp* transcription and are hence obvious candidates for being involved in their regulation (26). One of these, Irr, reacted particularly strongly (to temperature change) and was found to bind a peculiar promoter element (the H box [for *hbp* family box]) that is conserved among *Bartonella hbp* genes and several other virulence factors (autotransporters and OMP43). Because expression of Irr seemed to antagonize HbpC transcription, Irr was suggested to be a repressor of HbpC expression. Consistently, Irr overexpression yielded a bloodstream-like expression pattern of *hbp* genes and generally seemed to amplify the effects of temperature (26). Battisti et al. further reported a certain influence of the major virulence regulator BatR, which was confirmed in a subsequent study with *B. henselae* (347). Although this second study indeed reproduced the subgroups as identified in *B. quintana* when analyzing bacterial gene expression using the *B. henselae* Houston-1 *in vitro* infection model, HbpB and HbpC were upregulated in the course of infection, while HbpA and HbpD were downregulated (*B. henselae* lacks an apparent ortholog of HbpE) (347). Such a regulatory pattern seems counterintuitive when comparing it to the transcriptional data for the closely related *B. quintana* and indicates that *hbp* expression (and function) may be more complex than our current understanding.

Roles for the Hbps of the second subgroup in mammal host infection are strongly supported by phenotypic data (see above). Interestingly, the proteins of this subgroup seem to be structurally close, since an antibody raised against HbpA of *B. quintana* cross-reacted with HbpD and HbpE of the same species. The significance of the environmental stimuli simulated *in vitro* is confirmed by the finding that *B. quintana* infecting a rhesus macaque reproduced the "mammal-like" expression pattern (25). Consequently, those Hbps of the first subgroup very likely function predominantly in context of the arthropod vector. Although a contribution of HbpC seems very likely (it is more than 100-fold upregulated at 30°C compared to 37°C), the role of HbpB remains unclear. It was generally expressed at lower levels, reacted more weakly (hemin) or not at all (temperature) to the stimuli that were potent for HbpC, and has been reported to lack hemin binding properties (25). Furthermore, an insertion of approximately 500 base pairs makes HbpB larger than the other Hbps. This insertion is predicted to partition extracellularly and features tandem repeats as well as an abnormally high GC content, indicating that it may originate from lateral gene transfer (296). However, HbpB was the only hemin binding protein of *B. tribocorum* that was found to be essential for establishing bacteremia in the STM screen (374).

Potential antigenicity is an important feature particularly of surface-exposed proteins of bacterial pathogens and was assessed for the Hbps in several studies. Interestingly, HbpA of *B. henselae* seems to be immunogenic from live bacteria in rabbits (107), but firm evidence suggests the absence of an antibody response against any Hbp in cats seropositive for this species (84). Importantly, the latter finding was strongly supported by an independent study that tested a protein microarray of almost all predicted open reading frames (ORFs) of *B. henselae* against a large set of cat sera and failed to detect antibodies against any hemin binding protein (432). These results indicate that the absence of Hbp antigenicity may be part of *Bartonella* reservoir host adaptation or that events/strategies exclusive for the reservoir host could interfere with immune detection of the Hbps (e.g., downregulation of the humoral immune response). However, the hemin binding proteins of *B. quintana* were found to be immunogenic in human infections (43), but this species seems to be not yet fully adapted to its reservoir host (see above).

ABC systems. ATP binding cassette (ABC) systems are a superfamily of protein machineries that use ATP hydrolysis to energize the transmembrane passage of various substrates and were hence also called "traffic ATPases" (excellently reviewed in references 110 and 111). They share an architecture composed of two integral inner membrane domains and two periplasmic domains that typically constitute four independent polypeptide chains in ABC importers but are usually fused into one protein in exporters (111). Since ABC systems themselves cover translocation over only the inner membrane, transport across the outer membrane can be accomplished in different ways depending on the substrate. Importers usually rely on unspecific or specialized porins for substrates of small or moderate size, while very large or rare cargo is bound by high-affinity outer membrane receptors that also enable transport over the outer membrane. These particular receptors are typically powered by the TonB machinery, hence using the proton motive force stored over the inner membrane (314, 341). ABC exporters characteristically assemble a complex including the actual ABC transporter (paradigmatic *E. coli* HlyB) with a periplasmic membrane fusion protein (*E. coli* HlyD) and the outer membrane channel TolC, thus allowing one-step export in a mechanism referred to as type I secretion (reviewed in reference 186).

Several ABC import systems have been revealed to be essential for *Bartonella* virulence in the two STM screens. Not surprisingly, these comprise the uptake system for heme (HutABC/HmuV) and two iron uptake systems (FatBCD/CeuD and Sit/YfeABCD) whose role in the lifestyle of *Bartonella* had already been proposed before (25). The HutABC/HmuV system has been explored in more detail in *B. quintana* and is genetically associated with genes for a putative heme degradation enzyme (*hemS* [also known as *hmuS*]) and the *tonB* gene of the bartonellae (331).

Many other hits in the STM screens revealed that several ABC proteins involved in amino acid import are essential for virulence (374, 427), thus confirming the key role of amino acids as a carbon source for the bartonellae (85). In particular, the branched-chain amino acid import system (*liv* [for leucine-isoleucine-valine]) was required for establishing blood-stage infection in both screens. It is interesting in this context that other *Rhizobiales* drop biosynthesis of these particular amino acids during growth in bacteroids and then completely depend on their supply from the host plant ("symbiotic auxotrophy") (342, 343). However, one enzyme of the branched-chain amino acid synthesis pathway was also essential for virulence in *B. birtlesii* (427), indicating that not only import but also biosynthesis of these amino acids is necessary at one point of the infection. Additionally, several other ABC importers were also hits in the STM screens, for example, the *ugp* glycerol-3-phosphate importer in the *B. tribocorum* screen (374).

In contrast, no apparent ABC exporters were essential for *Bartonella* virulence, but exporters of all kinds are inherently prone to extracellular complementation which could render them difficult to detect in an STM screen. Thus, some ABC export systems of *Bartonella* may somehow participate in pathogenicity, although experimental evidence is lacking (see "Other virulence factors" below). However, it is pointless to speculate about the contribution of particular systems as long as their substrates have not been identified. Type I secretion systems (T1SS) in other pathogens are known to secrete various virulence factors, such as adhesins (156) or surface layer proteins (416).

In addition to import or export functions, a third subgroup of ABC cassette proteins has been implicated in other contexts, e.g., UvrA as a prominent component of the DNA excision repair machinery (160). Similarly, the ChvD ABC protein is known to be involved in the regulation of virulence gene expression in *A. tumefaciens* (263) and was found to be essential for pathogenicity in the *B. tribocorum* STM screen (374), indicating that the *Bartonella* orthologs also contribute to the infection process.

Apart from ABC systems, other transporters were also revealed to be critical for *Bartonella* virulence in the STM screens (374, 427). One of them, the *pha* multicomponent K^+/H^+ antiporter, was shown to be crucial for pH adaptation and a host-associated lifestyle in other *Rhizobiales* (345).

Other virulence factors. Many studies have discovered other virulence factors of *Bartonella* that cannot be reasonably sorted into one of the categories used in this review or for which substantial knowledge on their function has not yet been determined.

The BatR/BatS two-component system was shown to be a master regulator of *Bartonella* virulence that guides the expression of a large regulon comprising various virulence genes and is induced at the slightly basic pH of human blood (347). Remarkably, a huge set of orthologous systems play key roles in the host interaction of other *Rhizobiales* (example in reference 28), and it is apparent that this vertically inherited transcriptional controller has assimilated horizontally acquired genes such as those coding for the VirB/D4 machinery into its regulon in *Bartonella*. Like the BatR/BatS system, other transcriptional regulators have been hit in the STM screens and may thus participate in coordinating the expression of the huge arsenal of *Bartonella* virulence factors. One of those, RosR (374), is well known to be essential for productive host interaction in other *Rhizobiales* such as *Sinorhizobium* (207).

OMP43 and OMP89, two outer membrane proteins of *Bartonella*, have been identified as fibronectin binders in addition to trimeric autotransporter adhesins and HbpA (108). The surface display of fibronectin binding proteins is a common theme among the infection strategies of many bacterial pathogens (reviewed in reference 175) and has been linked to endothelial invasion and inflammation, for example, in infective endocarditis (346) as a well-known complication of *Bartonella* infections. OMP43 of *Bartonella* is a porin-like protein homologous to OMP2b of *Brucella* (which is known to be a potent inhibitor of apoptosis [248]) and further exhibits considerable sequence similarity to the RopA protein of *Rhizobium leguminosarum*, whose expression is strongly repressed in bacteroids (65, 125). In addition to fibronectin binding, OMP43 was shown to bind HUVECs and thus was proposed to serve as a major adhesin during *Bartonella* infection (65, 162). The promoter of the *omp43* gene contains an H box that is indicative of transcriptional control by Irr (26), and a study of *B. henselae* found that OMP43 expression was downregulated during infection of endothelial cells *in vitro* (347). OMP89 is the *Bartonella* homolog of BamA/YaeT, the major component of the Gram-negative outer membrane protein assembly/biogenesis machinery (reviewed in reference 231). No further evidence beyond its property to bind fibronectin would indicate a direct role of this protein in *Bartonella* pathogenesis. Consistent with their localization on the cell surface, both OMP43 and OMP89 are highly immunogenic in infections with *Bartonella* (43, 84).

Amazingly, it is apparent that the bartonellae encode the full machinery required for synthesis and secretion of cyclic β-(1,2)-glucans, i.e., the respective synthetase (ChvB in *Agrobacterium tumefaciens* [79], BH00920 in *B. henselae*) and the dedicated ABC exporter (ChvA in *A. tumefaciens* [70], BH10210 in *B. henselae*) (6, 34). Cyclic β-(1,2)-glucans are circular oligosaccharides known to enforce the host interaction of both symbiotic and pathogenic *Rhizobiaceae* (comprehensively reviewed in reference 48). In *Brucella*, another genus of zoonotic pathogens within this taxon, cyclic β-(1,2)-glucans contribute to bacterial surface integrity and are essential to prevent lysosome fusion of the *Brucella*-containing vacuoles by somehow manipulating lipid rafts in host cells (14, 58). Despite the function of *Brucella* cyclic β-(1,2)-glucans in subverting defenses of a vertebrate host, the *B. abortus* cyclic β-(1,2)-glucan synthetase was sufficient to complement accordant mutants in *Rhizobium meliloti* as well as *A. tumefaciens* and restored the associated phenotypes (196), confirming that cyclic β-(1,2)-glucans are highly conserved among *Rhizobiales*. Although the presence of cyclic β-(1,2)-glucans has not been specifically examined for *Bartonella*, the conservation of the dedicated machinery and the abundance of these compounds as rhizobial host-interacting factors make it very likely that (i) *Bartonella* synthesizes cyclic β-(1,2)-glucans and (ii) they somehow play a role in *Bartonella*-host interaction. No gene related to cyclic β-(1,2)-glucans was found to be essential for virulence in the *Bartonella* STM screens (374, 427), but extracellular complementation of the secreted compound may have impeded detection. Consistently, none of the various STM screens in *Brucella* brought up any gene linked to cyclic β-(1,2)-glucans (except for one intergenic hit more than 200 base pairs downstream of an associated succinyl-transferase gene) (144, 188, 255, 256), although they are known to be essential for intracellular survival of this pathogen (14).

Like *Brucella*, the bartonellae are known to evade the endocytic pathway and to establish a perinuclear niche inside their host cells (see above). Work with *B. henselae* showed that the bacterial factor(s) orchestrating this process is independent of both BadA and the VirB/D4 T4SS (246). Instead, a small-scale evaluation of transposon mutants revealed three peculiar proteins and HbpD as essential for this process. Although the contribution of these factors is not understood on a mechanistic level, their role in phagosomal escape could be confirmed by complementation analysis.

CONCLUDING REMARKS

It is striking that the leading symptoms of Carrion's disease, such as immune-mediated hemolytic anemia or pathological angiogenesis, are generally absent in reservoir host infections by the modern species but may occasionally arise in infections particularly of immunocompromised individuals and/or in incidental hosts. We therefore conclude that the bacterial factors responsible for these symptoms may be conserved over the whole genus *Bartonella*, especially when considering the virtual absence of large disparities in the arsenal of known virulence factors and the overall congruent course of infection. However, the activities of these factors are apparently tightly regulated and balanced with opposing activities and host responses in the modern species, thus allowing them to establish a controlled, silent course of infection that avoids elicitation of an immune response and favors vector transmission. Correspondingly, quiescent infections with persistent, asymptomatic bacteremia are prevalent in a certain proportion of individuals colonized by *B. bacilliformis*, confirming that the morbidity associated with Carrion's disease is not inherent to the infection cycle of this species.

We are convinced that the acquisition of type IV secretion systems in ancestors of the modern bartonellae, thanks to the highly modular nature of their virulence functions, opened up the opportunity to evolve a versatile cocktail of both cooperating and antagonistic activities for particularly fine-tuned host interaction. Decreased morbidity in combination with evolutionary versatility and at least partial redundancy of virulence functions may then have promoted host adaptability and thus the evolutionary success of the modern bartonellae. We find our hypothesis supported by the fact that the two adaptive radiations in lineages 3 and 4 were triggered only after the evolution of a diverse set of VirB/D4 effector proteins and the duplication and diversification of extracellular parts of the Trw T4SS.

The comprehensive listing and discussion of current knowledge on the molecular pathogenesis of *Bartonella* has demonstrated that the fragmentary and isolated nature of most knowledge on these remarkable pathogens is a major obstacle to drawing a coherent model of what underlies the diseases we observe in human patients. We thus recommend that future research should aim at combining our knowledge from cellular, clinical, microbiological, and veterinary research as well as the various models and strains used in the field. It seems particularly important to link the data obtained *in vitro* from infections with the Houston-1 and Marseille strains of *B. henselae*, but also with *B. quintana*, since these species are the most common cause of human *Bartonella* infections around the world and may serve as useful models for the other modern species. Furthermore, a comparison to the knowledge available on infections with *B. bacilliformis* seems desirable, especially in order to reveal the molecular mechanisms underlying its human host restriction, which may help to understand the zoonotic potential of *Bartonella*.

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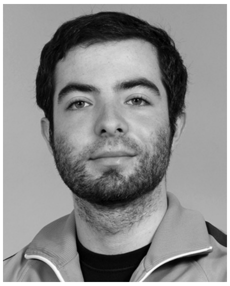

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